Microbiological workflows for the detection of Listeria monocytogenes in food

by Rolf Ossmer, Merck Millipore, with Volker Lanz and Andreas Bubert.

Listeriosis, which is caused by eating food contaminated with the bacterium Listeria monocytogenes (L. monocytogenes), is an important public health concern globally.

The Center for Disease Control (CDC) estimates that approximately 1,600 illnesses and 260 deaths occur annually due to listeriosis in the US. Similarly, in Europe 1,470 human cases were reported in 2011 with a mortality rate of 12.7%. To ensure consumer safety, rapid detection methods of L. monocytogenes in food are critical. However, traditional methods for detection require an enrichment step which is time consuming and labour intensive.

This article describes a solution designed to simplify and shorten the workflow of pathogen enrichment. Readybag Half Fraser Broth (Merck Millipore) are aluminium pouches filled with pre-weighed and gamma-irradiated granulated culture media which eliminate the need for upfront media preparation. It is compliant with ISO standard formulation.

The article will evaluate the application of the enrichment media in Readybag pouches for the detection of pathogens in wiener sausages, cream cheese, cantaloupe melon and cooked prawns. It will provide the workflow and data for L. monocytogenes detection according to ISO 11290-1 in detail. Traditional preparation of Half Fraser Broth was used as the reference method.

Food type	Test strain (food isolates)	No. of samples	Half Fraser (positive results)	
			Readybag	Standard
Wiener sausage	L. mono St 26/1/II/03	10	9	10
Cream cheese	L. mono W29/1/II B 2002	10	9	10
Cantaloupe melon	L. mono W 07/13	10	10	9
Cooked prawns	L. mono Frd. Nr. 594	10	9	8

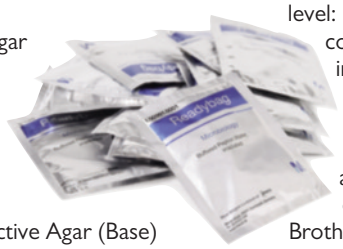
Table 1. Results of food sample testing using Readybag Half Fraser Broth and traditional Half Fraser Broth (autoclaved) according to ISO (11290-1) standard method.

Materials and equipment

Culture media:
- Readybag Half Fraser Broth 12.5g (1.02449.0060).
- FRASER Listeria Selective Enrichment Broth (base) (1.10398.0500).
- FRASER Listeria Ammonium iron (III) Supplement (1.00092.0010).
- FRASER Listeria Selective Supplement (1.00093.0010).
- Chromocult Listeria Selective Agar (base) (1.00427.0500).
- Chromocult Listeria Agar Selective Supplement (1.00432.0010).
- Chromocult Listeria Agar Enrichment Supplement (1.00439.0010).
- PALCAM Listeria Selective Agar (Base) (1.11755.0500).
- PALCAM Listeria Selective Supplement (1.12122.0010).

PCR test kits:
- Foodproof StarPrep II Kit (S 400 08, Biotecon Diagnostics GmbH, Potsdam).
- Foodproof Listeria monocytogenes Detection Kit (R 302 23, Biotecon).

Test strains:
- L. monocytogenes, St 26/1/II/03.
- L. monocytogenes, W29/1/II B 2002.
- L. monocytogenes, Frd. Nr. 594.
- L. monocytogenes, W 07/13.



Methods

The workflow for the food trials was based on ISO 11290-1. Four food types were investigated: wiener sausages, cream cheese, cantaloupe melon and cooked prawns, all sourced from a local supermarket. Each sample of mentioned foods was portioned aseptically to 25g, and the samples were added into sterile stomacher bags. All food samples were inoculated at low levels with 1mL of a L. monocytogenes suspension (inoculation level: 1-5cfu/25g sample). Negative controls were used without inoculation.

For the traditional workflow Half Fraser Broth was prepared by weighing, dissolving, autoclaving and aseptic addition of supplements. Half Fraser Broth (225mL) was added to each food sample. For the Readybag workflow, the pre-weighed and sterile content of one Readybag pouch was added directly to the food sample followed by the addition of sterile, demineralised water dispensed from an Elix Advantage Water Purification System. All samples were homogenised in a paddle blender for one minute. The stomacher bags were incubated at 30°C for 24 hours.

After 24 hours of incubation the Half Fraser Broth was streaked to Chromocult Listeria Agar and Palcam Agar, and 0.1mL of

the culture was transferred to 10mL of Fraser Broth. Chromocult Listeria Agar and Palcam Agar were incubated at 37°C and read out after 24 hours and – if necessary – after an additional 24 hours to check for the presence of characteristic colonies.

Fraser Broth was incubated at 37°C for 48 hours and streaked to Chromocult Listeria Agar and Palcam Agar. Both agar media were incubated at 37°C and checked for characteristic colonies after 24 hours and 48 hours. Characteristic colonies from the plates were confirmed by real-time PCR.

Results

All spiked samples of wiener sausage, cream cheese, cantaloupe melon and cooked prawns showed positive results with both the traditional and Readybag workflows. There were no false-negative results (see Table 1). All negative controls without spiking showed no characteristic colonies on the agar plates. There were no false-positive samples with either of the workflows.

Additionally, testing of salmonella with Readybag Buffered Peptone Water against the traditional autoclaved media according to ISO 6579:2002 led to comparable results (data not shown; workflow is similar to that of Half Fraser Broth).

Conclusion

Food trials with the enrichment of Listeria monocytogenes demonstrate that the Readybag Half Fraser Broth workflow provides results identical to the traditional workflow with media preparation in an autoclave. Readybag pouches offer significant time savings compared to the traditional method of sample preparation.

In the case of listeria enrichment with Half Fraser Broth, using Readybag pouches reduced workflow time (media preparation to incubation) from 24 minutes to eight. ■

References are available from the author on request