Application Note

Readybag® Half Fraser Broth – Compliance with ISO standard

Listeria monocytogenes is one of the most dominant foodborne pathogens worldwide. Testing for this parameter as described in the ISO standard referenced below is time- and labor-consuming.

Readybags are a solution designed to simplify and shorten the workflow of pathogen enrichment. They are aluminum pouches filled with pre-weighed and gamma-irradiated granulated culture media which eliminate the need for upfront media preparation.

Readybag® Half Fraser Broth is compliant with ISO standard formulation.

- ISO 11290-1:1996/Amd 1:2004 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method. First step in this standard method is an enrichment of the food sample in Half Fraser Broth.

The aim of our study was to evaluate the application of the EMD Millipore enrichment media in Readybags for the detection of pathogens in certain food categories. As an example we are showing the workflow and data for *L. monocytogenes* detection according to ISO 11290-1 in detail. Traditional preparation of Half Fraser Broth was used as a reference.

Materials:

Culture Media	
	Readybag® Half Fraser Broth 12.5 g (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, Postsdam)
	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
Food samples	
	Wiener sausages
	Cream cheese
	Cantaloupe melon
	Cooked prawns

Equipment:

- Mettler Toledo PR5002 balance
- EMD Millipore Elix® Advantage Water Purification System with EPod and sterile filter
- Autoclave Systec VX-75
- Lab paddle blender: Masticator, IUL Instruments
- Lateral filter bag for blender: BBag – 03, sterile SAL 10-3, 190 mm x 300 mm, 400 ml
- Memmert incubator BS10 and BS5
- Stratagene Mx3005P, Agilent Technologies

Method:

Detection of *Listeria monocytogenes* in food samples (ISO 11290-1)

The workflow for the food trials was based on ISO 11290-1 (see Fig. 1) Four food types were investigated, Wiener sausages, cream cheese, Cantaloupe melón, cooked prawns.

Each sample of mentioned foods was portioned aseptically to 25 g, and the samples were added into sterile stomacher bags. All food samples were inoculated at low levels with 1 mL of a *L. monocytogenes* suspension (inoculation level: 1-5 cfu / 25 g sample). Negative controls were used without inoculation.

For the traditional workflow Half Fraser Broth was prepared by weighing, dissolving, autoclaving and aseptical addition of supplements (see Fig. 1). Half Fraser Broth (225 mL) 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, demineralized water dispensed from an Elix® Advantage Water Purification System (see Fig. 1).

All samples were homogenized in a paddle blender for 1 minute.

The stomacher bags were incubated at 30°C for 24 h.

After 24 hours of incubation the Half Fraser Broth was streaked to Chromocult® Listeria Agar and Palcam Agar, and 0.1 mL of the culture was transferred to 10 mL of Fraser Broth.

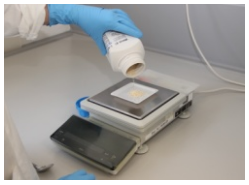
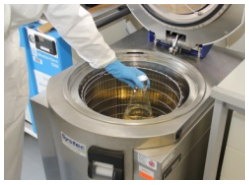
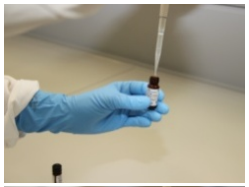
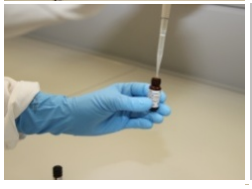
Chromocult® Listeria Agar and Palcam Agar were incubated at 37°C and read out after 24 h and – if necessary – after a further 24 h to check for the presence of characteristic colonies.

Fraser Broth was incubated at 37°C for 48 h and streaked to Chromocult® Listeria Agar and Palcam Agar. Both agar media were incubated at 37°C and checked for characteristic colonies after 24 h and 48 h.

Characteristic colonies from the plates were confirmed by real-time PCR.

25 g of food sample + 225 mL of Half Fraser Broth

24 h ± 2 h at 30 °C

Traditional workflow		Readybag® workflow
	Weighing of 12.5 g of dehydrated Fraser Listeria Selective Enrichment Broth (base)	No activity
	Transferring of defined amount of Fraser Listeria Selective Enrichment Broth (base)to a vessel	No activity
	Adding 225 mL of demineralized water	No activity
	Dissolving	No activity
	Autoclaving	No activity
	Cooling down to room temperature	No activity
	Adding Fraser Listeria Selective Supplement	No activity
	Adding Fraser Listeria Ammonium iron (III) Supplement	No activity

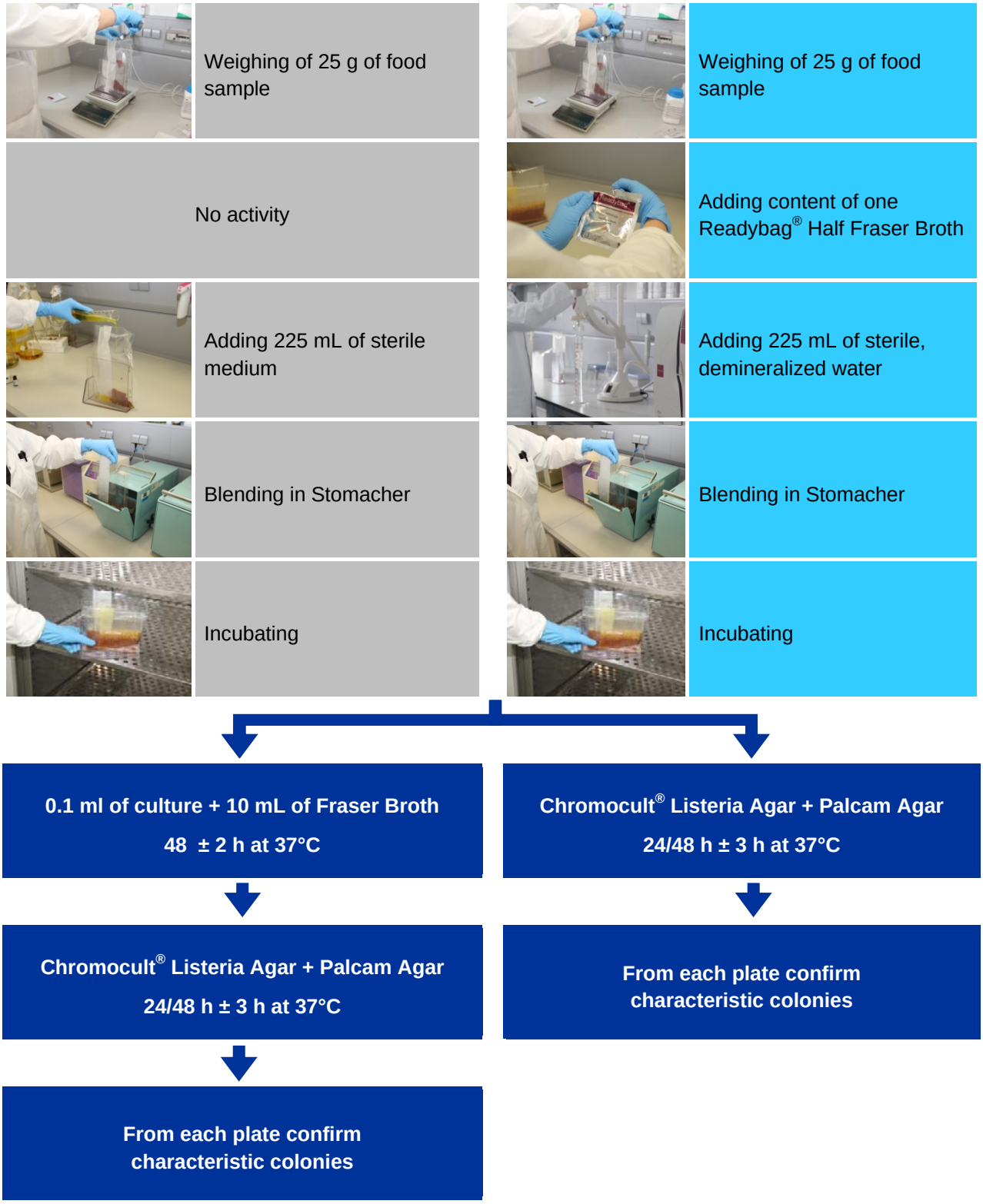


Fig. 1. Detection of *Listeria* – Comparison of workflow according to ISO 11290-1 and Readybag® workflow

Results:

Detection of *Listeria monocytogenes*

All spiked samples of Wiener sausage, cream cheese, Cantaloupe melon, and cooked prawns showed positive results with both workflows, Readybag® workflow and traditional workflow. There were no false-negative results.

All negative controls without spiking showed no characteristic colonies on the agar plates. There were no false-positive samples with either of the workflows.[1]

Food type	Test strain	Number of Samples	Readybag® Half Fraser (positive results)	Standard Half Fraser (positive results)
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

Tab. 1. Readybag® Half Fraser Broth – Internal Food Trials: Results of food sample testing using Readybag® Half Fraser Broth and traditional Half Fraser Broth (autoclaved) according to ISO standard method (ISO 11290-1)

[1] Testing of Salmonella with Readybag® Buffered Peptone Water compared to the traditional autoclaved media according to ISO 6579:2002 led to comparable results. The data is not shown here as the workflow is almost similar to that of Half Fraser Broth.

Conclusion:

Culture media preparation with Readybag® granulated media pouches is easy and convenient, and there is no need for additional equipment. The content of one bag can be added directly to the food sample plus sterile water. During the blending procedure the culture medium will be completely dissolved. Therefore, culture media consumption does not need to be planned in order to prepare the required quantities upfront, the user can rather start directly with the food sample. Readybags have a shelf life of three years without the requirement of cool storage, they are ready at hand at all times and do not take much space on the lab bench. This leads to an enormous gain in flexibility.

The shortening of the workflow with Readybag® compared to the traditional workflow leads to considerable time savings. In the case of *Listeria* enrichment with Half Fraser broth the working time of our lab technicians could be reduced from a total of 24 minutes to only 8 minutes with Readybags.

Food trials with the enrichment of *Listeria monocytogenes* could demonstrate that the Readybag® Half Fraser Broth workflow showed results identical to the traditional workflow with media preparation in an autoclave. Gamma-irradiated media with purified water from an Elix Advantage Water Purification System have thus proven equivalence to autoclaved culture media.

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