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Application Note

Evaluation of the stability of Half Fraser broth prepared from gamma-irradiated Readybag® Half FRASER pouches

Analysis of broth made from Readybag® Half FRASER pouch stored for different lengths of time at either 5°C or 25°C before inoculation.

Listeria monocytogenes is one of the most dominant foodborne pathogens worldwide. EN ISO 11290-1:1996 + Amd 1:2004 and EN ISO/DIS 11290-1:2014 describe the test method for the detection and enumeration of *Listeria monocytogenes*. The selective primary enrichment broth used for this is Half FRASER Broth, which contains half of the concentration of acriflavine and nalidixic acid in comparison to FRASER broth. Half FRASER Readybag® culture media is EN ISO 11290-1:1196 + Amd 1:2004 and EN ISO/DIS 11290-1:2014 compliant and is quality controlled by an ISO 17025 accredited laboratory. Readybag® media is a granulated culture media, provided in prefilled, gamma irradiated pouches with a three-year shelf life. The food testing routine is simplified because all that is required is the addition of sterile water before use. This procedure removes the need to autoclave the culture medium, which is the most time-consuming step in media preparation.

According to ISO 11133:2014 section 4.4.2.1 and 4.4.2.2, prepared media can be stored at 5°C ± 3°C and should be checked after defined storage times for their physical, chemical and microbiological performance characteristics. The aim of this study was to evaluate the influence of storage time and temperature of prepared Readybag® Half FRASER broth on its stability of productivity, selectivity and sterility. Inoculation was carried out at 0, 3, 6, 9, 24, 48, 72 and 96 hours after media preparation. Using sterile water (autoclaved), prepared Readybag® Half Fraser samples were stored at 5°C, and at 25°C. Each case was tested in duplicate and negative controls were done.

The evaluation was performed by the Institute of Veterinary Food Science – Department of Veterinary Medicine, Justus Liebig University of Giessen, Germany, utilizing stability trials.

Method:

Table 1: Culture media and supplements used for stability trial

Product Name	Cat. No.
Readybag® Half FRASER (Demi FRASER) Broth with Supplements acc. ISO 11290, 12.5 g, irradiated	**102449**
GranuCult™ Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM (TSA)	**105458**
ReadyPlate™ CHROM Listeria Agar acc. OTTAVIANI and AGOSTI acc. ISO 11290*	**146186**
GranuCult™ Sodium Chloride Peptone Broth (Buffered) acc. EP, USP, JP	**110582**
GranuCult™ BHI (Brain Heart Infusion) Broth acc. ISO 6888	**110493**

*According to ISO 11290-1:1996 + Amd 1:2004 the name ALOA is commonly used. In the following, it is named Listeria Selective Agar.

All products were obtained from Merck KGaA, Darmstadt, Germany and Merck Life Science GmbH, Eppelheim, Germany.

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Table 2: Readybag® Half FRASER broth stability trial test performance specification

Test strain	Inoculum (cfu/25 mL)	Incubation (cfu/10 µl) *	Specification	Reference
Mix culture: *Listeria monocytogenes* WDCM 00109 (ATCC® 35152) *Escherichia coli* WDCM 00013 (ATCC® 25922) *Enterococcus faecalis* WDCM 00087 (ATCC® 29212)	10-100 $>10^3$ $>10^3$	22 h at 30°C	>10 *L. mono* colonies	Listeria Selective Agar
Escherichia coli WDCM 00013	$>10^3$	26 h at 30°C	No growth	TSA
Enterococcus faecalis WDCM 00087	$>10^3$	26 h at 30°C	<100	TSA

*For easier demonstration, the specification was extrapolated to cfu/mL in the following.

- Specifications and *E. coli* + *E. faecalis* strains were chosen according to ISO 11133:2014 Table E.
- The stability trial includes negative controls (not inoculated samples), samples with mixed culture (*Escherichia coli, Enterococcus faecalis* and *Listeria monocytogenes*) and each a sample with the test strains *Escherichia coli* and *Enterococcus faecalis*.
- For preparation of a working culture, one colony was inoculated to 5 mL BHI broth and incubated for 18-24 hours at 35-37°C.
- After a serial dilution diluted with 9 mL Sodium Chloride Peptone to a certain end point cfu, 25 mL Sodium Chloride Peptone were inoculated with test strains (**Table 2**).
- Readybag® Half FRASER broth was prepared with 225 mL of demineralized, autoclaved water and inoculation was carried out after 8 different storage times (after 0, 3, 6, 9, 24, 48, 72 and 96 hours) at two different temperatures: 5°C in a refrigerator and 25°C in an incubator to simulate room temperature.

Physical, chemical and microbiological performance characteristics

Before inoculation of prepared Readybag® pouch followed by incubation at 30°C for 22 hours (mix culture; see **Table 2**) and at 30°C for 26 hours (*Escherichia coli, Enterococcus faecalis* and negative controls), all samples were examined optically to test for turbidity, color and precipitation and pH value. The mix culture was used to test the productivity of Readybag® pouch, therefore the shortest incubation time regarding ISO 11290-1:1996 was used; to test selectivity and sterility of the medium, samples of *E. coli, E. faecalis* and negative controls were used at the longest incubation time. All samples were double tested.

10 µl of each mix culture was spread out on either Listeria Selective Agar and incubated at 37°C for 44 hours, or on TSA with incubation at 37°C for 24 hours for the negative control, *E.coli* and *E. faecalis* cultures in accordance with ISO 11290-2 AMD. The criteria were more than 10^3 cfu/mL of *L. monocytogenes* in the mixed sample, less than 10^4 cfu/mL of *E. faecalis* and no growth of negative control and *E. coli*.

Stability trial Readybag® Half FRASER broth

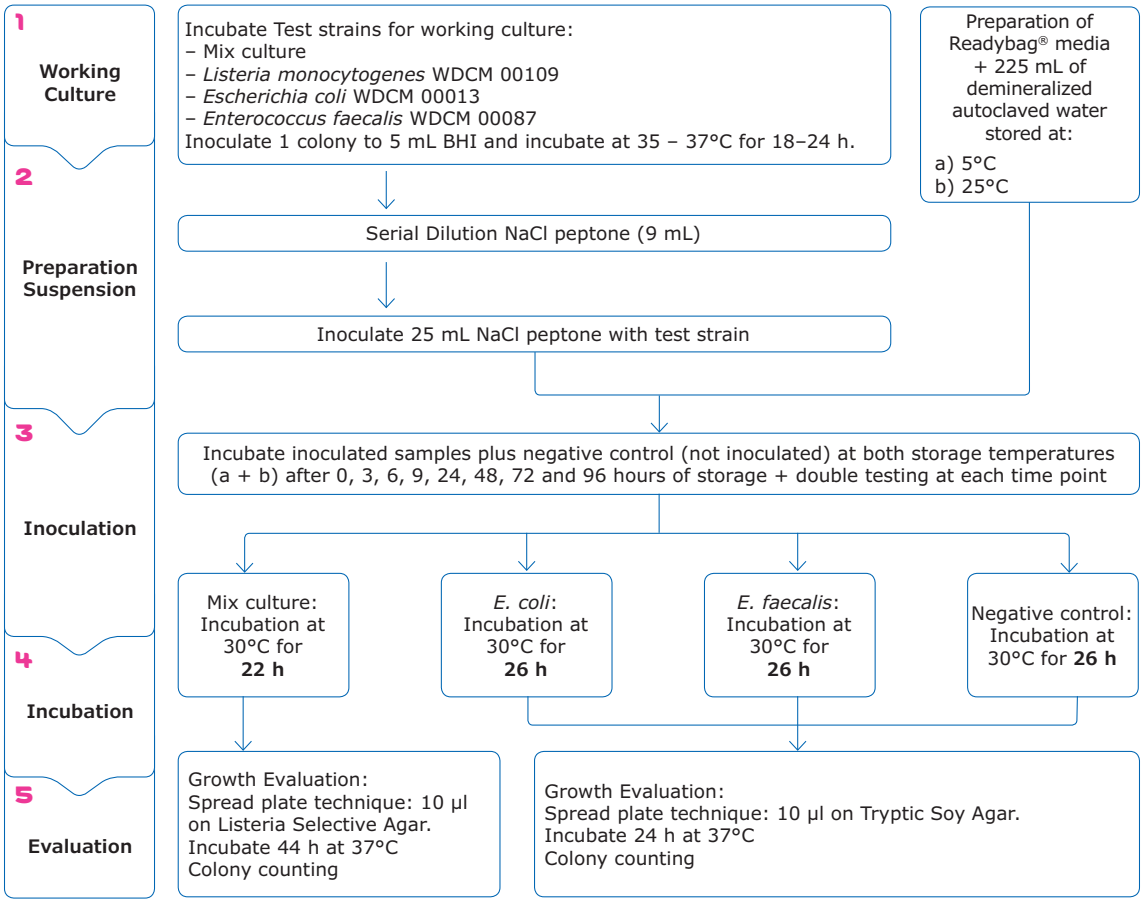

Figure 1: Workflow of stability trial for Readybag® Half FRASER broth at different storage times and temperature. Inoculation for *Listeria* was 10-100 cfu/25 mL and for *E. coli* and *E. faecalis* >10^3 cfu/25 mL.

Results:

Based on the results of stability trials, Readybag® Half FRASER broth could be stored up to 96 h at 5 °C as well as at room temperature at 25°C. This was documented by 8 different storage times (double testing), which were used for an inoculation of a mixture of *E. coli, E. faecalis* and *L. monocytogenes*, a negative control, an inoculation of *E. coli* as well as an inoculation of *E. faecalis* (inoculation levels see **Table 2**). After storage, just before inoculation, all samples were controlled optically (no turbidity or precipitation was shown and the color was light yellow) and the pH value was measured (see **Table 3**).

Table 3: pH measurement of stability trials for Readybag® Half FRASER broth

Storage time (h)	Storage at 5 °C	Storage at 25°C
0	7.13	7.13
3	7.17	7.15
6	7.17	7.16
9	7.14	7.14
24	7.16	7.16
48	7.16	7.14
72	7.14	7.14
96	7.16	7.15

All negative controls showed no growth of any microorganisms independent of the tested storage time and temperature.

Evaluation of mix culture

The productivity for *Listeria monocytogenes* in prepared Half FRASER broth was tested in a mix culture with *E. coli* and *E. faecalis*, incubated at 30°C for 22 h. All samples were inoculated according to the specification; except three* had an inoculation level less than 10 cfu/25 mL, but even these low inoculated samples were enriched to more than 103 cfu/mL (**Table 4**). Samples at all storage times independent on tested temperature fulfilled the specification according to ISO 11133.

Table 4: Inoculated samples with mix culture: results for productivity test of *L. monocytogenes*

Storage time (h) double testing	Inoculum (cfu/ 25 mL)	Growth after 5°C storage Results (cfu/mL)	Growth after 25°C storage Results (cfu/mL)
0*	10 – 100	$>10^3$ – $\leq 10^4$	$>10^3$ – $\leq 10^4$
3			
6			
9*			
24			
48			
72*			
96			

*One sample out of the double testing at each marked time point was inoculated with less than 10 cfu/ 25mL.

Evaluation of *Escherichia coli*

All samples were inoculated, according to specification, with *E. coli* and incubated for 26 h at 30°C. As no samples showed growth of *E. coli*, specifications according to ISO 111333 were fulfilled. There was no loss of selectivity even at long storage times with no cooling of the prepared Readybag® Half FRASER broth (**Table 5**).

Table 5: With *Escherichia coli* inoculated samples for selectivity test for Readybag® Half FRASER broth

Storage time (h) double testing	Inoculum (cfu/ 25 mL)	Growth after 5°C storage Results (cfu/mL)	Growth after 25°C storage Results (cfu/mL)
0	$>10^3$ – $>10^4$	No growth	No growth
3			
6			
9			
24			
48			
72			
96			

Evaluation of *Enterococcus faecalis*

E. faecalis was inoculated with $>10^3$ cfu/ 25mL. The criteria according to ISO 11133 was no growth or growth less than 10^4 cfu/mL after 26 h at 30°C of incubation, spread out on TSA. All cases were double tested. None of the samples, which were stored at 5 °C, showed any growth of *E. faecalis*. Just three samples of the storage at 25 °C allowed a reduced growth of *E. faecalis* with 1 cfu/10µl. Remaining samples also showed no growth (**Table 6**).

Table 6: With *Enterococcus faecalis* inoculated samples of stability trials for Readybag® Half FRASER broth

Storage time (h) double testing	Inoculum (cfu/ 25 mL)	Growth after 5°C storage Results (cfu/mL)	Growth after 25°C storage Results (cfu/mL)
0	$>10^3 - >10^4$	No growth	No growth
3			
6*			
9			
24			
48*			
72			
96*			

*One sample out of the double testing at each marked time point at 25°C showed a reduced growth of *Enterococcus faecalis*.

Interpretation:

The high productivity of Readybag® Half FRASER broth for *Listeria* was demonstrated by using a mix culture for inoculation. *L. monocytogenes* were efficiently enriched according to the specification. Readybag® Half FRASER broth also demonstrates an excellent selectivity for *E. coli* and *E. faecalis*, although the inoculation dose of the named bacteria was very high. Neither storage time up to 96 hours nor temperature (5°C or 25°C) had an influence on the selectivity or productivity of Readybag® Half FRASER broth. There was no change of pH value and no growth on negative controls during storage time, independent of the temperatures tested. In addition, there were also no changes in the observed physical, chemical and microbiological characteristics.

Broth medium prepared from irradiated Readybag® Half FRASER pouches and stored for up to 96 hours even at room temperature can be used for *Listeria* detection according to EN ISO 11290-1:1196 + Amd 1:2004 and EN ISO/DIS 11290-1:2014. All test strains fulfilled the specification and ISO 11133 criteria.

A storage time up to 96 hours even at room temperature reduces waste and costs because prepared media does not need to be used immediately, which reduces waste. Readybag® Half FRASER pouch is very easy to use due to simply adding sterile water obtained by autoclaving or from commercially available automated water purification systems, to the product, there is no need for autoclaving and weighing which saves a significant amount of time.

Literature:

1. ISO International Standardisation Organisation: Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method – Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data. EN ISO 11290-1:1996 + Amd 1:2004.
2. ISO International Standardisation Organisation: Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and other Listeria spp. – Part 1: Detection method. EN ISO/DIS 11290:2014.
3. ISO International Standardisation Organisation. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. ISO 11133:2014

Further Reading:

Application Note: Evaluation of irradiation influence of culture media on growth promotion. Comparative analysis of half-FRASER Readybag® broth with non-gamma irradiated half-FRASER GranuCult™ media

Technical Data Sheet: Readybag® Half FRASER (Demi FRASER) Broth with Supplements acc. ISO 11290

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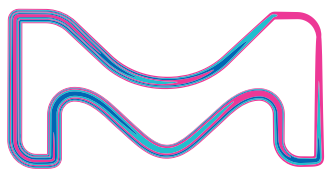

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