

Evaluation of the stability of Buffered Peptone Water prepared from gamma-irradiated Readybag® Buffered Peptone Water pouches.

Analysis of broth made from Readybag® Buffered Peptone Water at different storage times at 5°C and 25°C before inoculation.

Salmonella is one of the major food-borne pathogens. EN ISO 6579:2002 and EN ISO 6579-1:2017 describe the test method for detection of *Salmonella* spp. in food, animal feed and environmental samples from the primary production by using Buffered Peptone Water (BPW) as the non-selective pre-enrichment liquid culture medium. The formulation and the specification for testing Readybag® BPW is compliant with EN ISO 6579:2002 and EN ISO 6579-1:2017. Readybag® pouches are pre-weighed and gamma-irradiated and have a shelf life of up to 3 years. The food testing routine is simplified because all that is needed is the addition of sterile water before use. This procedure avoids the need to autoclave the culture medium, which is the most time-consuming step in media preparation.

According to ISO 6579-1:2017, prepared media can be stored for 72 hours at 5°C, but according to ISO 11133:2014 section 4.4.2.2, media should be checked to establish the expiry date after defined storage times to assess the physical, chemical and microbiological performance characteristics.

The aim of this study was to evaluate the influence of storage time and temperature of prepared Readybag® BPW on its stability of productivity and sterility. Inoculation was carried out at 0, 3, 6, 9, 24, 48, and 72 hours after media preparation. Using sterile water (by autoclaving), prepared Readybag® BPW samples were stored at both 5°C, and 25°C. Each experiment was carried out in duplicate along with relevant negative controls.

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® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP, 5.7 g, irradiated	102448
GranuCult™ Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM (TSA)	105458
GranuCult™ Sodium Chloride Peptone Broth (Buffered) acc. EP, USP, JP	110582
GranuCult™ BHI (Brain Heart Infusion) Broth acc. ISO 6888	110493

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

Table 2: Readybag® Buffered Peptone Water stability trial performance specification

Test strain	Inoculum (cfu/25 mL)	Incubation	Specification (cfu/mL)	Reference
<i>Salmonella</i> Typhimurium WDCM 00031 (ATCC® 14028)	10 – 100	18 h at 37°C	>10 ⁷	TSA
<i>Salmonella</i> Enteritidis WDCM 00030 (ATCC® 13076)	10 – 100	18 h at 37°C	>10 ⁷	TSA

- Test strains and incubation conditions were chosen according to ISO 11133:2014, Table E.
- The possibility to store prepared Readybag® BPW up to 72 hours was evaluated at two different temperatures (5°C in a refrigerator and 25°C in an incubator to simulate room temperature).
- ISO 11133:2014 recommends incubating test strains for 18-24 hours in a non-selective broth. For preparation of a working culture, one colony was used to inoculate 5 mL BHI broth and then incubated for 18-24 hours at 35-37°C.
- After a serial dilution with 9 mL Sodium Chloride Peptone, the test strains were inoculated in 25 mL Sodium Chloride Peptone.

Physical, chemical and microbiological performance characteristics

The stability trial includes negative control, and samples of each *Salmonella* Typhimurium and *Salmonella* Enteritidis at inoculation levels according to specification (see **Table 2**) to test the productivity of *Salmonella*. Readybag® BPW prepared with 225 mL demineralized and autoclaved water was tested at a range of 7 storage times: Inoculation was carried out at 0, 3, 6, 9, 24, 48 and 72 hours after media preparation. The test strains were inoculated in 25 mL Sodium Chloride Peptone.

Before inoculation, in addition to testing of the pH value, the turbidity, color and precipitation of each sample was also measured using optical methods. Measurements were taken in duplicate.

The inoculated samples and in parallel the negative control were incubated at 37°C for 18 hours according to specification, and EN ISO 6579:2002. After a serial dilution with 9 mL Sodium Chloride Peptone broth, the samples were then spread out on Tryptic Soy Agar (TSA), and incubated at 37°C for 24 hours.

Stability trial Readybag® Buffered Peptone Water

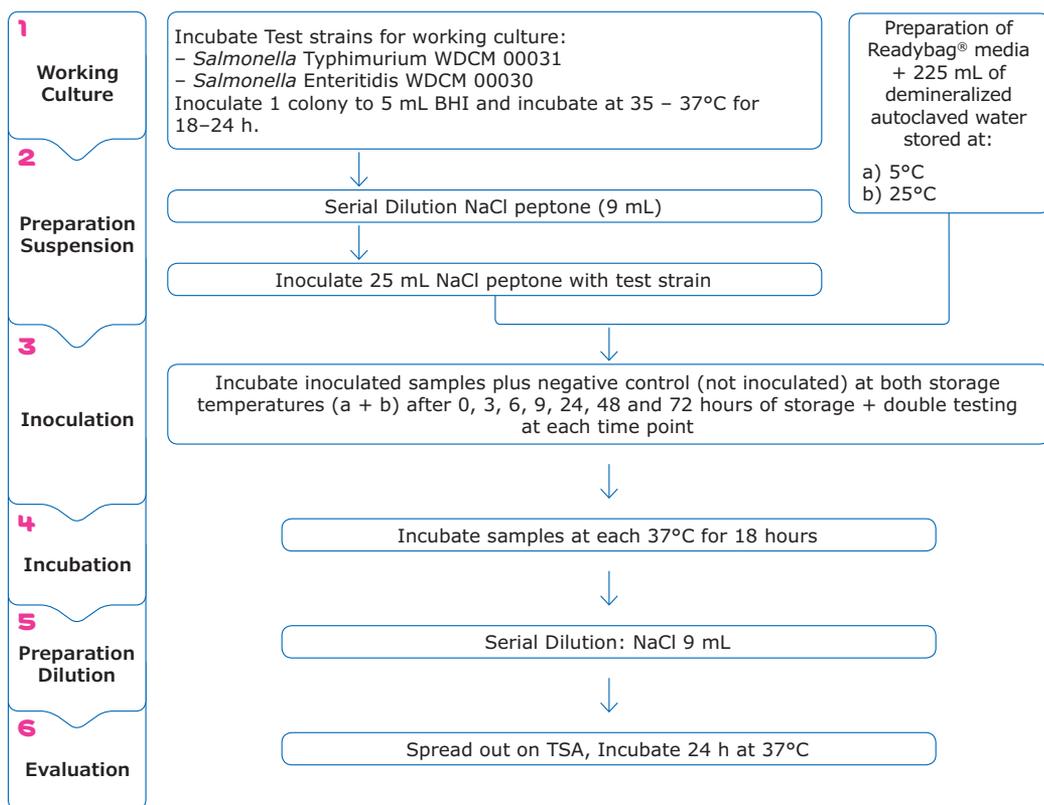


Figure 1: Workflow of stability trial for Readybag® BPW at different storage times and temperature. Inoculation for both *Salmonella* strains was 10 – 100 cfu/25 mL.

Results:

Based on the results of stability trial, prepared Readybag® Buffered Peptone Water could be stored for up to 72 h independent of temperature. In these trials, 7 different storage times (double testing) were used for a not inoculated sample (negative control), samples with *S. Typhimurium* WDCM 00031 as well as *S. Enteritidis* WDCM 00030 (both 10 – 100 cfu/25 mL). After storage, just before inoculation, all samples were subjected to optical control (no turbidity or precipitation was shown and the color was light yellow) and the pH value was measured (Table 3).

Table 3: pH measurement of stability trial of Readybag® Buffered Peptone Water

Storage time (h)	Storage at 5 °C	Storage at 25°C
0	7.07	7.07
3	7.04	7.09
6	7.12	7.13
9	7.03	7.13
24	7.11	7.09
48	7.09	7.04
72	7.12	7.09

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

Evaluation of *Salmonella Typhimurium*

Each sample was double tested and inoculated, according to specifications, with 10-100 cfu/25 mL. The goal was a quantitative result of $>10^7$ cfu/mL. Although two samples were inoculated with less than 10 cfu/25 mL, all samples had a result of $>10^8 - \leq 10^9$ cfu/mL, independent of the tested temperature, which exceeds the specification of $>10^7$ cfu/mL (Table 4).

Table 4: Inoculated samples with *S. Typhimurium* of Readybag® Buffered Peptone Water stability trial

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			
3*			
6*			
9	10 – 100	$>10^8 - \leq 10^9$	$>10^8 - \leq 10^9$
24			
48			
72			

*One sample out of the double testing was inoculated with less than 10 cfu/25 mL.

Evaluation of *Salmonella Enteritidis*

Each sample was double tested and *S. Enteritidis* was inoculated according to specifications, with 10-100 cfu/25 mL. Although two samples were inoculated with less than 10 cfu/25 mL all samples had a result of $>10^8 - <10^9$ cfu/mL, independent of the tested temperature, which exceeds the specification of $>10^7$ cfu/mL (Table 5).

Table 5: Inoculated samples with *S. Enteritidis* of Readybag® Buffered Peptone Water stability trial

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			
3*			
6*			
9	10 – 100	$>10^8 - <10^9$	$>10^8 - <10^9$
24			
48			
72			

*One sample out of the double testing was inoculated with less than 10 cfu/25 mL.

Interpretation:

According to ISO 6579-1:2017, prepared media can be stored up to 72 hours at 5°C. The evaluation confirms that prepared Readybag® Buffered Peptone Water could not only be stored for 72 hours at 5°C but also at room temperature. No negative influence on productivity, sterility or pH value was observed. Both strains of *Salmonella* fulfilled the specification.

A storage time up to 72 hours even at room temperature reduces waste and costs because prepared media does not need to be used immediately, which reduces waste.

Readybag® Buffered Peptone Water 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 of 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 of *Salmonella* spp. EN ISO 6579:2002.
2. ISO International Standardisation Organisation. Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. EN ISO 6579-1:2017.
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 and buffer capacity. Comparison analysis of gamma-irradiated Buffered Peptone Water (BPW) Readybag® with non-gamma irradiated BPW GranuCult™

Technical Data Sheet: Readybag® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 2964, FDA-BAM and EP

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