Millipore®
Preparation, Separation,
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Application Note

Evaluation of irradiation influence of culture media on growth promotion and buffer capacity

Comparison analysis of gamma-irradiated Readybag® Buffered Peptone Water (BPW) with non-gamma irradiated GranuCult® BPW

Salmonella is one of the major a 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.

GranuCult® Buffered Peptone Water and Readybag® culture media are EN ISO 6579 and EN ISO 6579-1:2017 compliant. The GranuCult® product is granulated and supplied in 500 g packs, while the Readybag® product contains the same granulated culture medium, but provided in gamma irradiated pouches prefilled to either 29.0 g or 86.0 g, which are adjusted to the testing food sample size. GranuCult® BPW needs to be autoclaved before use. Instead, as Readybag® BPW pouches are pre-weighed and gamma-irradiated, the need for upfront media preparation such as weighing and autoclaving is eliminated and the food testing routine is simplified because sterile water is just added before use. The three year shelf life of Readybag® BPW pouches compared to three months for self-prepared media, reduces costs and waste associated with media storage.

According to EN ISO 6579 and EN ISO 6887-1, the pH of the pre-enrichment should not fall below 4.5 if using acidic food. Therefore buffer capacity of BPW is important to maintain the pH during the enrichment step.

The scope of the comparison study was to demonstrate that gamma-irradiation (10-20 kGray) of culture media has no influence on the growth promotion of *Salmonella* and the buffer capacity. GranuCult® Buffered Peptone Water was used as a reference for a non-gamma-irradiated dehydrated culture media.

The evaluation study on a growth influence of irradiation was performed by the Institute of Veterinary Food Science – Department of Veterinary Medicine, Justus Liebig University of Giessen, Germany, utilizing growth promotion tests and food trials.

Method:

Product Name	Usage	Cat. No.
Readybag® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP, 5.7 g, irradiated	GPT, FT & BC	~~1024480060~~*
GranuCult® Buffered Peptone Water (BPW) acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	GPT, FT & BC	**107228**
GranuCult® RVS (RAPPAPORT-VASSILIADIS-Soya) Broth (Base) acc. ISO 6579	FT	**107700**
GranuCult® MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579	FT	**105878**
Rambach Agar (Ready-to-use plates)	FT	**146719**
GranuCult® XLD (Xylose Lysine Deoxycholate) agar acc. ISO 6579	FT	**105287**
Singlepath® Salmonella	FT	**104140**
GranuCult® Tryptic Soy agar acc. EP, USP, JP, ISO and FDA-BAM	GPT & FT	**105458**
GranuCult® BHI (Brain Heart Infusion) broth acc. ISO 6888	GPT & FT	**110493**
Sodium chloride peptone broth (buffered)	GPT & FT	**110582**

Table 1. Culture media and supplements used for growth promotion tests (GPT), food trials (FT) and buffer capacity (BC)

* The article 102448 Readybag Buffered Peptone Water 5,7 g, irradiated, was discontinued in 2019. Other Readybag® Buffered Peptone Water products (Cat. No. 1.00908 29.0 g, irradiated, and Cat. No. 101865 86.0 g, irradiated) are available.

The documented test results are still valid because the composition ingredients were not changed.

Test strain	
Salmonella Typhimurium WDCM 00031	Official culture collection test strains; Included in performance testing for quality assurance in EN ISO 6579 and EN ISO 6579-1:2017
Salmonella Enteritidis WDCM 00030	

Table 2. Test strains

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Growth Promotion Test

The incubation at 37 °C (± 1) for 18 h (± 2) for the primary enrichment was chosen according to ISO 6579 and ISO 6579-1:2017.

The shortest (16 h) and longest (20 h) incubation time were selected to evaluate the final concentration of colony forming units (cfu) per sample. Growth promotion data were collected for both tests strains (**Table 2**) and repeated 10 times.

The initial inoculation level (cfu/per sample) was determined by plating out on Tryptic Soy Agar (TSA, **Figure 1**).

Readybag® and GranuCult® BPW media were inoculated with the same suspension to ensure comparability and the tests were performed in parallel.

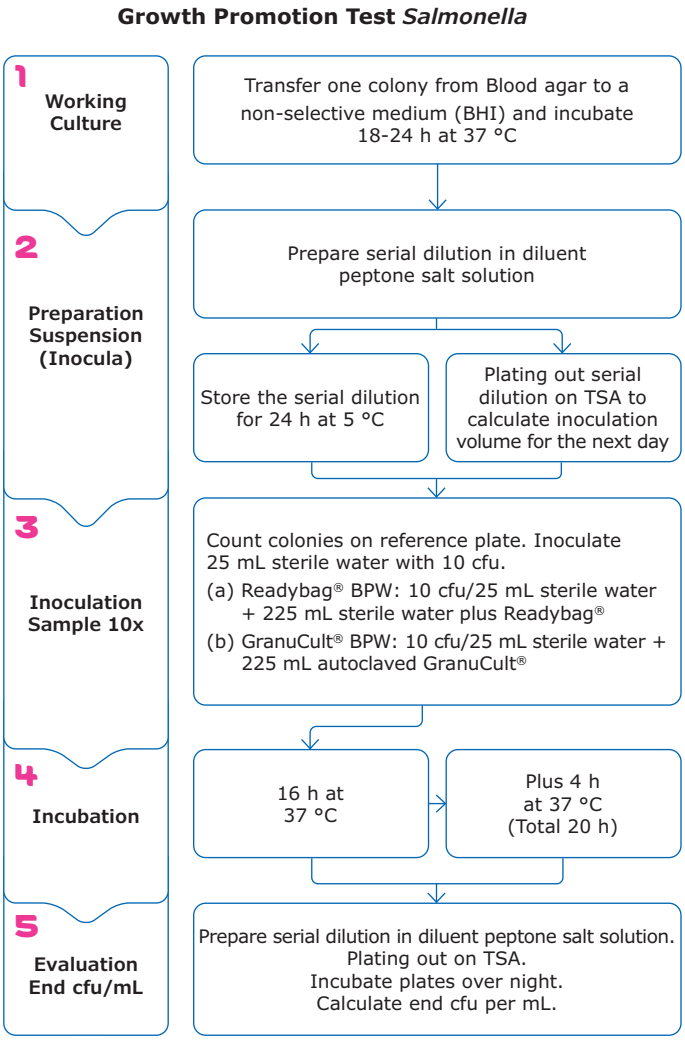

Figure 1. Workflow Growth Promotion Test. The growth promotion test was repeated 10 times per strain.

Food Trials

Fresh chicken meat was used to evaluate growth promotion in presence of a food matrix. Spiking was conducted as described above using *Salmonella* Typhimurium WDCM 00031 (1cfu/sample) for Readybag® and GranuCult® BPW enrichment media. RVS and MkTTn were chosen as secondary enrichment broths and XLD and Rambach Agar as selective plating media.

For comparison, the inoculation and detection of *Salmonella* Typhimurium in food matrix was performed in parallel with Readybag® and GranuCult® BPW culture media using the same bacterial suspension. A total of 20 food samples were inoculated.

The food matrices were inoculated with 1 colony forming unit and the level was controlled by plating out on TSA (**Figure 2**).

Singlepath® Salmonella was used for confirmation of presumptive colonies. This step was done once per sample and per plating medium after the RVS enrichment step.

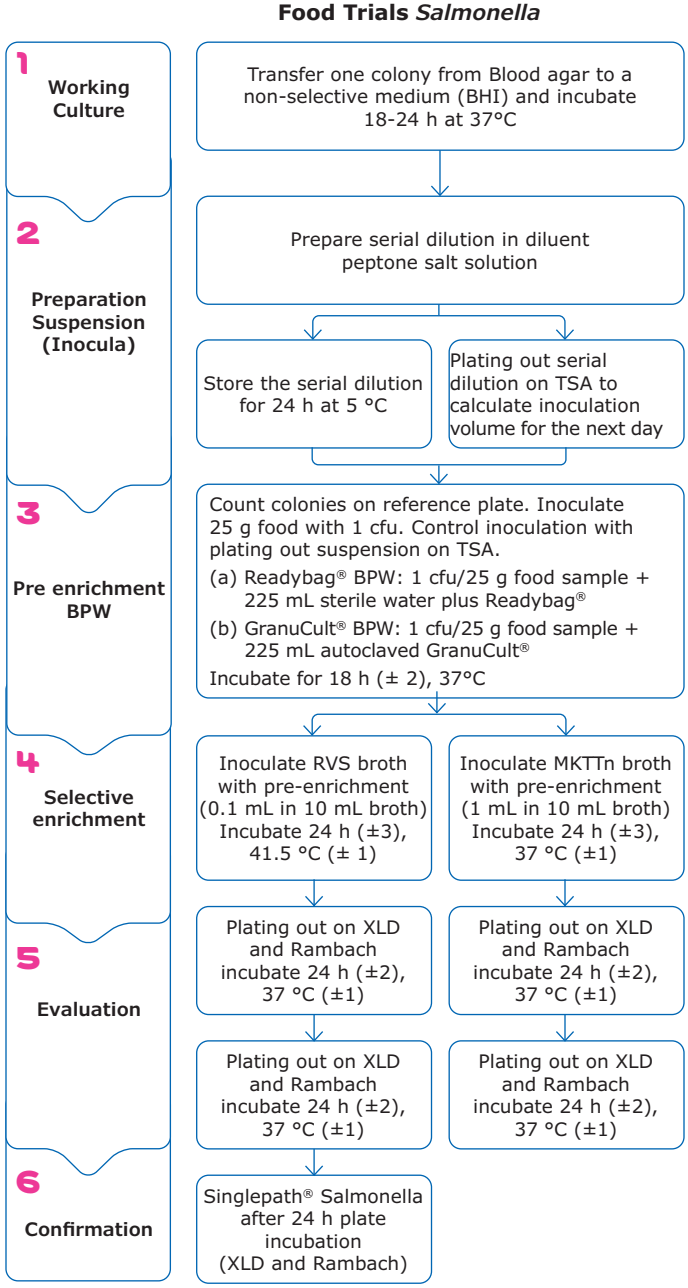

Figure 2. Workflow analyses of food spiked with *Salmonella* Typhimurium and *Salmonella* Enteritidis. According procedure for *Salmonella spp*. detection in food production according to EN ISO 6579-1:2017.

A well-isolated, suspect colony was picked from the selective plate (XLD and Rambach) and re-suspended in 200 µL RVS broth. The test procedure was according to Singlepath® Salmonella protocol specified in the product insert.

For each trial one negative control and one positive control (10^2) were included. The food sample was not naturally contaminated.

Results:

Growth Promotion Test

The results of the growth promotion tests are shown in **Figure 3** and **4**. Growth curves are based on the median values of 10 results per test strain and sampling time point. The 25th percentile (lower quartile) and 75th percentile (upper quartile) are marked.

All samples showed growth of the target bacteria. The slope of the curves between 16 h and 20 h incubation is highly similar indicating an equal growth rate.

The tests confirmed no measurable influence of gamma-irradiation on growth promotion.

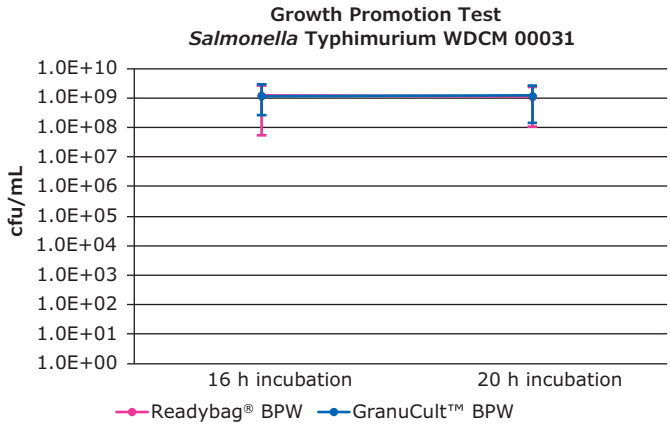

Figure 3. Growth Promotion Test *Salmonella* Typhimurium WDCM 00031. Colony forming units after 16 and 20 h incubation

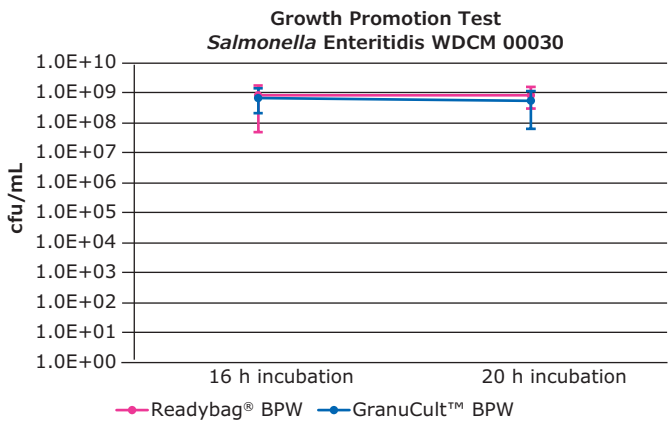

Figure 4. Growth Promotion Test *Salmonella* Enteritidis WDCM 00030. Colony forming units after 16 and 20 h incubation

Food Trials

The results of the food sample testing (n=20) are summarized in **Table 3**. The table shows the positive and negative results of culture media evaluation after 24 h plate incubation (XLD and Rambach agar) and the sensitivity of Singlepath® Salmonella.

The detection rate of positive and negative samples is in line with those obtained from the MkTTn enrichment.

Food Matrix: Chicken	Positive	Negative	Total Sample	Sensitivity Singlepath® Salmonella
Readybag® BPW	12	8	20	100%
GranuCult® BPW	14	6	20	100%

Table 3. Food Trial Results *Salmonella* Typhimurium WDCM 00031, Inoculation Level 1 cfu; Results using RVS or MKTTn broth and between XLD and Rambach agar as selective plating medium were comparable.

Buffer Capacity

BPW provides good conditions for recovery and cell growth. The buffer components should prevent the shift of the pH-value during growth under pH 4.5 to avoid false-negative results. The target organism *Salmonella* usually stop growing at this low pH condition.

Figure 3 shows the average pH-values out of three titrations per product. A total volume of 18 mL 1.0 N HCl was added to 225 mL BPW.

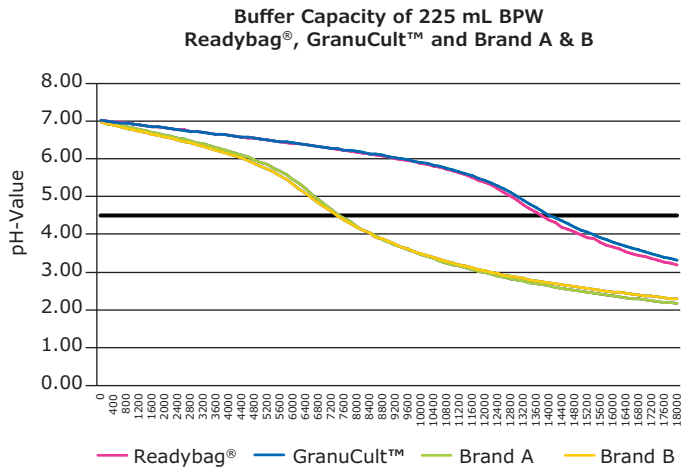

Figure 5. Buffer capacity of buffered peptone water Readybag®, GranuCult®, Brand A and Brand B. The black line marks pH 4.5.

According to ISO 6579 the pH-value should not fall below 4.5. Readybag® and GranuCult® reach the 4.5 value after an addition of 13.5 – 14 mL of 1.0 N HCl. While competitor brands A & B reach the ISO cut of level already after the addition of 7.4 mL.

An influence of gamma-irradiation on buffer capacity was not detectable compared to non-gamma-irradiated GranuCult®. Readybag® and GranuCult® BPW showed the best buffer capacity.

Interpretation:

The external evaluation study investigated the potential impact of gamma irradiation on growth promotion by using two different *Salmonella* strains.

GranuCult® BPW and irradiated Readybag® BPW culture media were inoculated at low levels with 10 cfu. Both ISO 6579 and ISO 6579-1:2017 describe a time frame of 18 h (± 2) for the primary incubation step. After 16 h and after 20 h of incubation both products show similar rate of growth. The slope of the growth curves is equal. The study revealed no detrimental effects on the growth promotion for *Salmonella* Typhimurium and *Salmonella* Enteritidis.

The food trial using chicken meat were conducted at a low level spiking of 1 cfu and gave comparable results according to ISO 6579 included confirmation of presumptive colonies with Singlepath® Salmonella.

Readybag® and GranuCult® BPW showed the best buffer capacity in the comparison study. This was approved by a titration with increasing addition of 1.0 N HCl.

The results showed that almost twice the amount of HCl was needed to reduce the pH of both products below pH 4,5 compared to the competitor products. This could have a cost saving impact when acidic foods below 4,5 need to be analysed.

The gamma-irradiation of Readybag® BPW has no significant negative influence on growth promotion and buffer capacity. This was documented by growth promotion tests, food trials and a pH titration test.

GranuCult® BPW and irradiated Readybag® BPW culture media can be used for *Salmonella* detection according to EN ISO 6579 and EN ISO 6579-1:2017.

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 spp*. - Part 1: Detection of *Salmonella spp*. EN ISO 6579-1:2017.
3. ISO International Standardisation Organisation. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions. EN ISO/FDIS 6887-1:2016.
4. ISO International Standardisation Organisation. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 4: Specific rules for the preparation of miscellaneous products. EN ISO/FDIS 6887-4:2016.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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Lit. No. AN1285ENEU Ver. 1.1
36218
05/2021