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Application Note

Comparative Performance Evaluation of Readybag® Buffered Peptone Water

Efficiency of Readybag® BPW usage and comparison with other buffered peptone water media for compliance, *Salmonella* spp. growth and buffering capacity

Introduction

There is an increasing demand in microbiological food and water testing for culture media that meet international regulatory standards such as ISO, EP and FDA-BAM.

Readybag® Buffered Peptone Water (Readybag® BPW), available in pouches, is a cost-effective granulated culture medium for detecting *Salmonella* spp. and other microorganisms in food samples. It is in full compliance with the regulatory standards ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP.[1,2,3,4,5]

Four *Salmonella* spp. strains were used for the tests. *Salmonella* spp. are gram-negative enteric pathogens and a leading cause of gastroenteritis, with outbreaks often linked to contaminated food. The most frequently used method for detecting *Salmonella* in food is based on the ability to recover even low numbers and sub-lethally injured cells. However, *Salmonella* growth and their fermentative metabolic processes during enrichment set acids free, adding to the acids that may be contained in the food sample. Acidity can impede further growth, so the media contain buffer components to prevent a downward shift of the pH-value. The greater the buffer's capacity, the better.

A further important quality feature of buffered peptone water (BPW) culture media is that it should enable an efficient growth rate during the exponential growth phase. This allows earlier sub-cultivation in selective enrichment media and facilitates subsequent molecular-based analyses.

This study comprises four separate comparisons of:

- The compliance documentation provided by the manufacturers of four BPW culture media and Readybag® BPW.
- The growth rates of *Salmonella* spp. when using Readybag® BPW and the four other brands of BPW to investigate which product enables the fastest growth.
- The buffering capacities of Readybag® BPW and the four other BPW brands.
- The workflow and cost efficiency of the Readybag® BPW method and two other methods in an independent investigation by Cherney Microbiological Services, Ltd.[7]

Material

Buffered peptone water products

Readybag® Buffered Peptone Water (Cat. No. 100908 29.0 g/pouch and 101865 86.0 g/pouch) and four other commercially available buffered peptone water products (brands A to D) were used for the lab tests.

Test strains

Four *Salmonella* spp. strains frequently associated with food contamination and illness were selected for the tests:

Salmonella Typhimurium ATCC® 14028™ / WDCM 00031

Salmonella Typhimurium ATCC® 13311™ / WDCM 00121

Salmonella Enteritidis ATCC® 13076™ / WDCM 00030

Salmonella Infantis ATCC® 51741™

The test strains were inoculated at low levels of 10 to 30 colony forming units (CFUs) per sample, similar to those found in contaminated food samples.

Methods

Growth promotion

An early and efficient onset of bacterial growth in a culture medium indicates that the microorganisms adapt rapidly to the culture medium. The slope of the curve gives a very good indication of the medium's growth performance capability. The steeper the curve, the more cells are available for detection at a given time. Growth performance was determined in two ways: turbidimetric measurement and the drop plate method.

Turbidimetric measurement: Readybag® BPW and the media brands A to D were inoculated with the *Salmonella* spp. strains at low levels and enriched, followed by automated turbidity measurements at 36.5 °C. The measuring interval was 20 minutes and the overall measuring period 20 hours.

Drop plate method[6]: 250 mL of BPW were inoculated with the same dilution of an overnight culture to ensure comparability, then enriched according to ISO 6579.[1] After enrichment, the samples were taken and dropped out on Columbia Blood Agar (Cat. No. 146559).

The turbidimetric measurements and the drop plate method were performed in parallel.

Buffer capacity

Titration in 250 mL of BPW: Readybag® and the four other BPW media (brands A to D) were prepared in accordance with manufacturer instructions. Measuring series were performed to determine the buffer capacity. The pH values were measured at t=0 and after every addition of 200 µL of 1N HCl until the acidity target (pH 2.0 to 3.0) was reached, which was when 18.0 mL had been added in total.

Workflow and cost efficiency

Workflow duration and other cost factors such as quality control testing, utility and storage space for the preparation of food samples were compared for the following three methods: the traditional method, which includes weighing and autoclaving of the BPW; the Readybag® method; and a high-efficiency, high-volume method comprising media and sample preparation. The high-efficiency method uses a sterilizer from which the BPW can be aseptically pumped into 20 L containers (carboys) which can then be transported to the lab area. The study examined the work hours required, the media costs and the utility and associated electricity costs for each of the three methods. The study was conducted at Cherney Microbiological Services, Ltd. USA.[7]

For each method, the hands-on time needed was recorded, as were the costs for preparing 50 semi-hard cheese samples weighing 375 g each and the associated media. Timing was stopped when the prepared samples were moved to be loaded into the incubator for enrichment. The food samples were not incubated or tested. Sample weighing and stomaching times were normalized as the processes were identical. Costs of labor, media, utilities, capital, and storage space were determined based on an average volume of 7,500 tests per month, the list prices of the media, and typical labor costs.

Results

Regulatory compliance

Table 1: Formulation, media preparation and QC compliance of Readybag® BPW and various competitor products (brands A-D) with regulatory standards for food

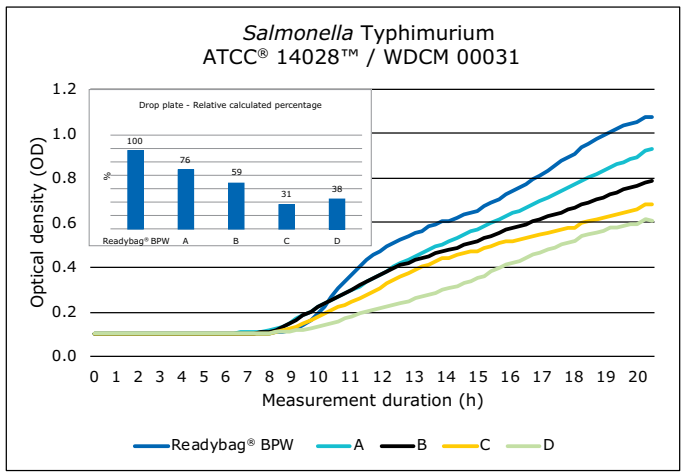
Compliance with standard	Readybag® BPW	A	B	C	D
ISO 6579-1:2017	√	x	x	√	x
ISO 21528-1:2017	√	x	x	x	x
ISO 22964:2017	√	x	x	x	x
FDA-BAM Method 5:2009	√	x	√	x	√
EP 10 chapter 2.6.31	√	x	x	x	x

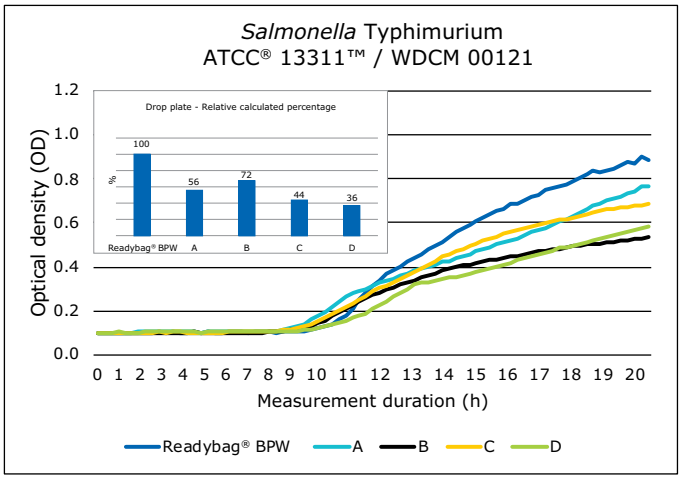
The comparison shows that only Readybag® is in compliance on formulation, media preparation, and quality control with all five regulatory standards. Three of the four other investigated products comply with only one food standard each and a further product complies with none of the standards at all.

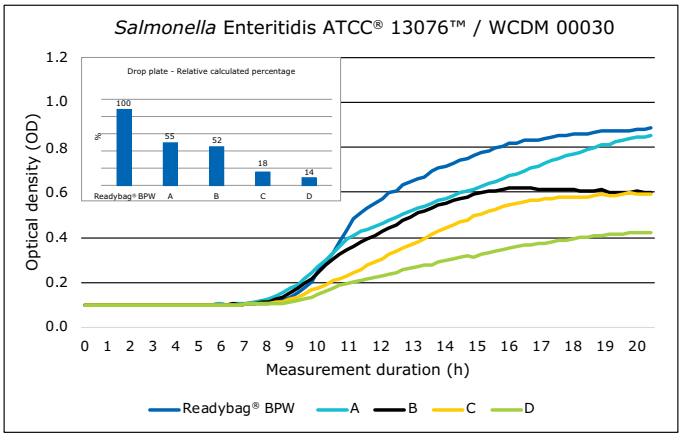
Growth promotion

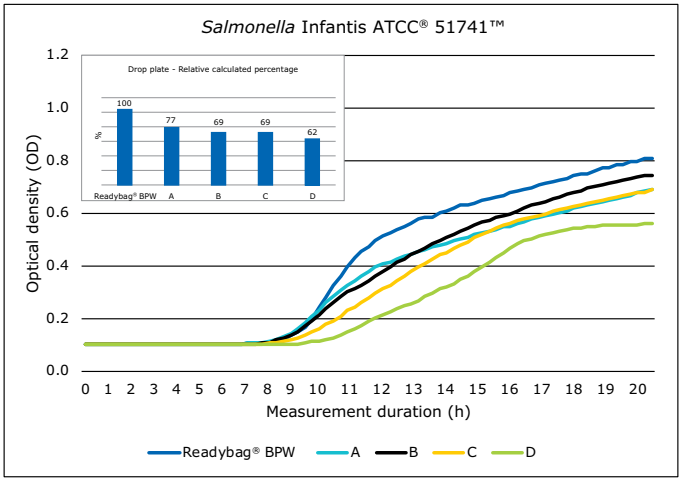
The growth of the four different *Salmonella* strains was measured turbidimetrically over 20 hours for all five buffered peptone water products.

Figure 1: Growth of *Salmonella* spp. in 5 different buffered peptone waters







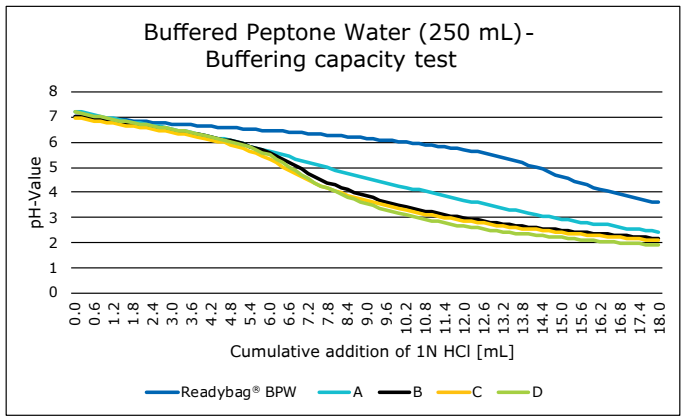


These results indicate that the most efficient growth was observed with Readybag® BPW. The *Salmonella* strains in all tested culture media started to grow at a similar time of between 7 and 9 hours after inoculation. However, Readybag® BPW led to the steepest slope in the exponential phase for all four test strains. For the drop plate method, the culture medium with the highest number of resulting CFUs per mL was set to 100% and the results of the other media calculated as relative percentages. The relative percentages for brands A to D were all well below the benchmark 100%. For each of the four test strains, these media provided significantly lower growth performance than the Readybag® BPW medium **(figure 1)**.

Buffer capacity and titration

The buffering capacity of buffered peptone water is important as acidic and acidifying foodstuffs can reduce the pH during the enrichment of samples. Many *Salmonella* species are unable to multiply at a pH of below 4.5. The buffering capacities of all five BPW products were tested by consecutively adding 1N HCl in 200 µL steps. The pH of 4.5 was taken as the critical value.

Figure 2: Buffering capacity test



The titration study revealed significant differences in the pH stability of the tested buffered peptone water products. Readybag® BPW displayed the best buffering capacity by being able to buffer a total added volume of about 15.2 mL in 250 mL of BPW, while the media A, B, C, and D reached the critical pH of 4.5 at lower volumes of 7.0 to 9.2 mL of HCl solution **(figure 2)**.

Workflow and efficiency

The cost comparison carried out by Cherney Microbiological Services, Ltd. of the three methods Readybag® BPW, High Efficiency, and Traditional included all handling steps associated with the analysis of the food sample. Besides media preparation, it also considered documented workflow duration and other cost factors such as quality control testing, utility and storage space for the preparation of food samples.

Table 2 lists the cost and times for all three methods, with the results taken from the Cherney Microbiological Services report published in a white paper.[10]

Table 2: Method comparison: Workflow and costs of preparing 50 semi-hard cheese samples (375 g each)
Costs (in USD) or time (in hours/days) by method

Cost and time parameters investigated	Readybag®	High Efficiency	Traditional
Labor cost	37.73	43.82	82.60
Utilities	1.35	14.90	37.58
Capital depreciation	3.91	27.00	9.39
Media cost	484.93	523.25	608.30
Footprint	0.00	0.13	0.10
Total cost	527.52	619.10	737.97
Prep.+ processing time in lab	2.7 h	3.1 h	5.9 h
Total elapsed time (sterlilization, autoclaving)	2.7 h	4 d	4 d

Conclusion

A comparative analysis of Readybag® BPW with the BPW from four other culture media manufacturers showed that only Readybag® BPW fulfills the regulatory requirements of the various considered ISO, FDA-BAM and European Pharmacopeia standards with respect to formulation, media preparation, and quality control.

In comparative growth performance tests, Readybag® BPW provided a steeper growth curve and a higher overall growth rate during the exponential growth phase. This feature enables the sub-cultivation in selective enrichment media at the earliest given time point as described in ISO 6579-1:2017 and FDA-BAM for testing *Salmonella* spp. The efficient growth of *Salmonella* spp. after 10 hours also makes Readybag® BPW attractive for testing combinations with molecular analyses. The molecular test is typically carried out at the earliest possible enrichment time. The growth promotion of *Salmonella* spp. in 250 mL of medium per flask led to a higher CFU/mL for Readybag® BPW than for brands A to D. Reaching a high CFU/mL in 250 mL is especially important for pathogen detection in food trials.

In the pH titration experiment, Readybag® BPW showed good buffering capacity, while the other BPW products displayed much lower capacities. Readybag® BPW needed significantly more addition of HCl to drop to the critical pH of 4.5. The pH stability of buffered peptone water is critical for enrichment in acidic foods such as berries and some cheese products. On the other hand, especially probiotic cultures such as lactobacilli and streptococci that are present in probiotic food and beverage samples (e.g. fermented dairy products including cheese, yogurt, ice cream, infant formula, but also fruit juices, sausages and cereal bars) can reduce the pH to below 4.5 during enrichment in buffered peptone water.[8] ISO 6887-1:2017 stipulates that double-strength buffered peptone water may then be used.[9] Due to its good buffering capacity, Readybag® BPW may still be used in single-strength concentration when the other four tested buffered peptone water products must already be applied in double concentration. This leads to cost savings in addition to those shown in the workflow efficiency analysis by the independent laboratory Cherney Microbiological Services, Ltd, USA.

The Cherney study's results showed that the Readybag® BPW method requires less overall labor than both the high efficiency and traditional methods when testing composite cheese samples. Especially for 375-gram samples, the Readybag® method features lower capital equipment costs for producing the needed sterile medium and a lower list price of the medium than the other two methods. Total costs were lower and preparation up to 3.2 hours faster. The time saving of using the Readybag® preparation method is mostly due to the avoidance of autoclaving or sterilization as needed for the preparation of BPW from the standard product. In conclusion, Readybag® saves time over the traditional method of sample preparation, and using these pouches decreases the risk of contamination when testing food samples.

Literature

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