# Optimized enrichment protocols to overcome *Salmonella* growth inhibition in various spices for detection with real-time PCR

Joshua Whitworth[1], Jennifer Pelowitz[1], Matthew Turner[2], Weijia Wang[2], Haiyun Wang[3], Jean-Philippe Tourniaire[4], Astrid Cariou[4], Christophe Quiring[4], and Sophie Pierre[4]
[1]Bio-Rad Laboratories, Hercules, CA
[2]Bio-Rad Laboratories, Singapore, Singapore
[3]Bio-Rad Laboratories, Beijing, China
[4]Bio-Rad Laboratories, Marnes-la-Coquette, France

## Introduction

Spice matrices introduce a variety of difficulties for the detection of *Salmonella* contamination. One important consideration to research before testing these foods is whether enrichment protocols combined with the spice allow *Salmonella* to grow to avoid false negative results. In this study, we investigated various enrichment combinations of buffered peptone water to overcome *Salmonella* growth inhibition in a variety of spices to allow detection using real-time PCR.

## Methods

### Enrichment Conditions and PCR Methods

Matrices were tested in triplicate with 2.5 or 5 g samples, with smaller sample sizes for higher dilutions. Samples were enriched with Bio-Rad Buffered Peptone Water Standard (BPW Standard, Bio-Rad Laboratories, catalog #12013259), with and without modifications, at various dilution ranges, with and without neutralizing additive, then spiked with < 10 CFU *Salmonella* Typhimurium ATCC 14028 (dry stressed) and incubated at 37°C for 22 ± 2 hr. After incubation, samples were extracted using the Easy I protocol and tested using the iQ-Check *Salmonella* II Kit (Bio-Rad Laboratories, catalog #3578123) using the FAST thermal protocol on a CFX96 Touch Deep Well Real-Time PCR Detection System. pH measurements were taken before and after incubation and all positive PCR results were confirmed by culture using Tetrathionate and Rappaport Vassiliadis broths as secondary enrichments followed by streaking to XLD and RAPID'*Salmonella* agars (Bio-Rad Laboratories, catalog #3563961).

### Test Treatments and Spices

Six spices were analyzed, and treatment conditions were based on FDA BAM and ISO protocol recommendations for the specific growth inhibitors contained in each spice (Table 1).

**Table 1. Summary of spices analyzed and test treatments for each sample.**

| Sample | Notes |
|---|---|
| Mixed Spice #1 | Acidic, multiples spices including garlic and chilis |
| Mixed Spice #2 | Sweet and sour spices including garlic, onion, and chilis |
| Mixed Spice #3 | Acidic, multiple spices including garlic and seaweed |
| Pure Spice #4 | Ground ginger |
| Mixed Spice #5 | Vanilla, possible high salt content |
| Pure Spice #6 | Ground garlic |

## Results

Successful growth of *Salmonella* was achieved in 24 hr with Mixed Spice #1 (BPW, increased enrichment ratio, data not shown), Mixed Spice #2 (BPW, standard enrichment ratio, optional supplement, data not shown), Mixed Spice #3 (Modified BPW, standard enrichment ratio, neutralizing additive, Table 2 and Figure 1), Mixed Spice #5 (BPW, increased enrichment ratio, data not shown), Pure Spice #4 (BPW, standard enrichment ratio, neutralizing additive, data not shown), and Pure Spice #6 (BPW, increased enrichment ratio, neutralizing additive, data not shown summarized in Table 3). All confirmation results matched PCR results (data not shown).

**Table 2. Example data from the Mixed Spice #3 test set.**

| Media | Dilution | Neutralizing Additive | PCR Positives | PCR Negatives |
|---|---|---|---|---|
| BPW | Standard | No | 0 | 3 |
| **BPW** | Standard | **Yes** | **3** | **0** |
| **BPW** | **> 1:10** | **No** | **3** | **0** |
| Modified BPW | Standard | No | 0 | 3 |
| **Modified BPW** | Standard | **Yes** | **3** | **0** |
| **Modified BPW** | **> 1:10** | **No** | **3** | **0** |

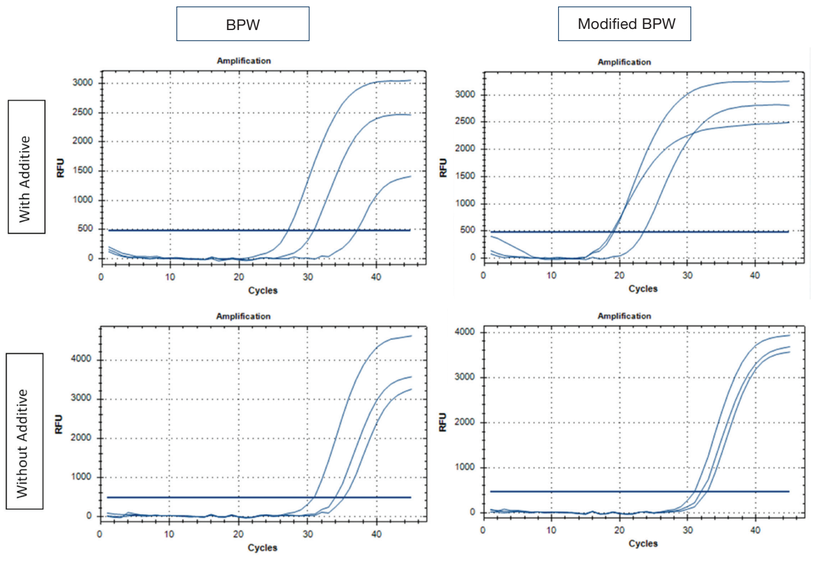


**Fig 1. Example data from Spice Mix #3 showing the effect of various treatments on PCR amplification curves.**

**Table 3. Summary of all spices tested with enrichment conditions balanced between detection optimization and cost-effectiveness.**

| Spice | Media | Dilution | Neutralizing Additive |
|---|---|---|---|
| Mixed Spice #1 | BPW | > 1:10 | No |
| Mixed Spice #2 | BPW | Standard | Optional |
| Mixed Spice #3 | Modified BPW | Standard | Yes |
| Pure Spice #4 | BPW | Standard | Yes |
| Mixed Spice #5 | BPW | > 1:10 | No |
| Pure Spice #6 | BPW | > 1:10 | Yes |

## Significance

These data show that *Salmonella* may not grow under standard enrichment conditions with some spices and universal enrichment protocols may not be appropriate for some spices. Labs may need to test and customize enrichments for specific spice mixes. This study demonstrated that custom enrichment protocols using Bio-Rad's BPW improved *Salmonella* growth in a cost-effective manner. In combination with the iQ-Check *Salmonella* II PCR detection kit, rapid detection of *Salmonella* in challenging spice matrices can be achieved.



IAFP 2023 TORONTO • ONTARIO Canada July 16-19

23-1144