

# Analysis of Pooled Environmental Sponges Using the iQ-Check *Salmonella* spp. Real-Time PCR Kit



Tech Note

## Introduction

Pooling, also referred to as wet pooling, is the combining of multiple samples post-enrichment into one sample to analyze using a rapid detection method. The advantage of pooling is that it can significantly reduce the costs per test while allowing the isolation of individual samples and sample locations for confirmation of presumptive positive results and corrective action investigations.

In this study we analyzed the performance of the iQ-Check *Salmonella* spp. real-time PCR kit when testing individual samples compared to pooled samples. Four groups of five environmental sponges were inoculated with various *Salmonella* serotypes and pooled post-enrichment to verify detection of *Salmonella* and general effects on assay performance.

## Methods

### Environmental Sample Preparation

Bacterial cultures were adjusted to achieve a target inoculum of ~10 CFU *Salmonella* and ~100 CFU competitor organisms. A volume of 100 µl of each organism was pipetted directly onto the environmental sponge surface (World Bioproducts LLC) with each *Salmonella* strain inoculated separately onto different sponges (Table 1). Cultures were also plated onto Tryptic Soy Agar (Hardy Diagnostics) for titer verification. Sponges were held overnight for 20–22 hr to equilibrate and were subsequently enriched with pre-warmed Buffered Peptone Water (Bio-Rad Laboratories) at 60 and 90 ml volumes.

**Table 1. Organism and inoculum levels used to contaminate select environmental sponges.**

Organism	Inoculum (CFU)
<i>Salmonella</i> Enteritidis	13
<i>Salmonella</i> Tennessee	2
<i>Salmonella</i> Typhimurium	19
<i>Salmonella</i> Newport	2
<i>Staphylococcus aureus</i>	10
<i>Enterococcus faecalis</i>	159

### Pooling and Testing

All sponges were incubated at  $37 \pm 1^\circ\text{C}$  for  $21 \pm 1$  hr and  $48 \pm 2$  hr and tested with the iQ-Check *Salmonella* spp. real-time PCR kit using the iQ-Check Prep automation system 15 ml tube workflow method. For individual sample results, one milliliter of each sample was transferred into separate sterile 15 ml tubes for testing per package instructions. For pooled testing, one milliliter from five different enrichments was combined into a 15 ml tube which created a grand total

of four pools. Each pool contained one inoculated sponge and four uninoculated sponges. Pools were mixed by pipetting up and down 20 times and then tested as a single sample. For the analysis of low levels of *Salmonella*, an aliquot of each of these individual and pooled sample tubes was serially diluted and tested using the iQ-Check *Salmonella* spp. real-time PCR kit and deep well plate workflow. Each dilution was subsequently plated onto RAPID'*Salmonella* chromogenic agar (Bio-Rad Laboratories) for enumeration.

## Results

Results (Cq values) are summarized in Table 2. A Cq value is the PCR cycle at which the fluorescence of the sample increases enough to cross the threshold line indicating a positive result. Pooled sample results had a Cq value 1-3 cycles later than the individual positive samples due to the dilution effect of the four negative sponges. This did not affect results as all inoculated samples were PCR positive and subsequently confirmed on chromogenic agar. In addition, all uninoculated samples had no growth when plated on chromogenic media. Samples were tested again after 48 hr of enrichment to assess the chance of pooled results changing after additional incubation and to assist some customers who may need to delay the testing of enrichments during weekends or holidays. Continuing sample incubation for an additional day did not affect *Salmonella* detection in this assay. Cq values remained nearly identical to those from the day before with no major improvement or reduction. Some customers may also use smaller enrichment volumes to save on media costs. Varying enrichment volumes had no effect on individual or pooled sample results in this test.

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**Table 2. Summary of iQ-Check *Salmonella* spp. PCR results comparing inoculated individual, uninoculated individual, and pooled samples.**

Pooling and Individual Test Results					
Organism	Preparation	20 hr		48 hr	
		60 ml Cq	90 ml Cq	60 ml Cq	90 ml Cq
<i>Salmonella</i> Enteritidis	Individual	20.34	20.67	20.45	21.27
	<b>Pool</b>	<b>21.81</b>	<b>21.43</b>	<b>22.33</b>	<b>23.1</b>
	Uninoculated	N/A	N/A	N/A	N/A
<i>Salmonella</i> Tennessee	Individual	20.49	20.18	20.03	20.48
	<b>Pool</b>	<b>22.21</b>	<b>21.99</b>	<b>21.68</b>	<b>22.34</b>
	Uninoculated	N/A	N/A	N/A	N/A
<i>Salmonella</i> Typhimurium	Individual	21.76	19.11	21.57	19.54
	<b>Pool</b>	<b>24.42</b>	<b>21.69</b>	<b>23.72</b>	<b>22.29</b>
	Uninoculated	N/A	N/A	N/A	N/A
<i>Salmonella</i> Newport	Individual	18.87	22.83	19.25	22.6
	<b>Pool</b>	<b>21.25</b>	<b>25.07</b>	<b>21.22</b>	<b>25.37</b>
	Uninoculated	N/A	N/A	N/A	N/A

This experiment along with previous internal testing has shown that the iQ-Check *Salmonella* spp. test kit can detect *Salmonella* concentrations as low as 10<sup>2</sup>–10<sup>3</sup> CFU/ml (Table 3). This concentration appears to adjust only slightly when pooling, changing to as low as 10<sup>3</sup> CFU/ml.

**Table 3. Summary of iQ-Check *Salmonella* spp. dilution series results of both inoculated individual and 5 sample pools**

Dilution Series					
CFU/ml	# POS	Avg Cq	CFU/ml	# POS	Avg Cq
Individual 90 ml			Individual 60 ml		
10 <sup>4</sup>	4	33.64	10 <sup>4</sup>	4	33.43
10 <sup>3</sup>	4	36.15	10 <sup>3</sup>	4	36.01
10 <sup>2</sup>	4	39.05	10 <sup>2</sup>	3	39.20
10 <sup>1</sup>	1	40.72	10 <sup>1</sup>	1	39.51
0–10 <sup>1</sup>	0	N/A	0–10 <sup>1</sup>	0	N/A
Pool 90 ml			Pool 60 ml		
10 <sup>4</sup>	4	34.30	10 <sup>4</sup>	4	34.64
10 <sup>3</sup>	4	37.89	10 <sup>3</sup>	4	37.89
10 <sup>2</sup>	2	39.51	10 <sup>2</sup>	1	39.99
10 <sup>1</sup>	1	40.2	10 <sup>1</sup>	0	N/A
0	0	N/A	0–10 <sup>1</sup>	0	N/A

**Conclusion**

Based on the results of this study, the iQ-Check *Salmonella* spp. real-time PCR kit is capable of detecting *Salmonella* concentrations of 10<sup>3</sup> CFU/ml or higher represented in a pool of five samples without compromising results compared to testing individual samples.

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