

Comparison Of The FDA BAM Salmonella Method And A New PCR Method For Detection Of Salmonella Species In Spices

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Overview

Purpose: The performance of the Thermo Scientific™ SureTect™ Salmonella species PCR Assay (Figure 1) was compared to the FDA-BAM method with dried powdered chilli, onion and garlic.

Methods: Replicates of 375 g of dried powdered chilli, onion and garlic were diluted 1:10 in Tryptone Soya Broth (TSB) ± 5 g/L Potassium Sulphite (K₂SO₃). The samples were pH adjusted as required and samples were spiked as shown in Figure 2. The SureTect Salmonella species PCR Assay followed by culture confirmation was performed after 20-22 hours incubation at 35°C. Additionally a panel of 24 exclusivity strains were tested using the SureTect Salmonella species PCR Assay.

Results: All SureTect Salmonella species PCR Assay positive results were correctly confirmed by culture. No false positive results were observed from either the unspiked samples and all exclusivity isolates panel.

Introduction

An evaluation of the SureTect Salmonella species PCR Assay was undertaken with dried powdered chilli, onion and garlic. Samples of 375g were diluted 1:10 in enrichment broth according to the FDA BAM, Chapter 5¹. Samples of 375g were tested to mimic the 15 samples of 25g that customers routinely pool into one homogeniser bag. Homogeniser bags containing spice samples were spiked, pH adjusted and incubated at 35°C for 20-22 hours. An exclusivity panel of organisms frequently isolated from these matrices was selected.

Figure 1: The Thermo Scientific SureTect Real-Time PCR Instrument and Kits



Methods

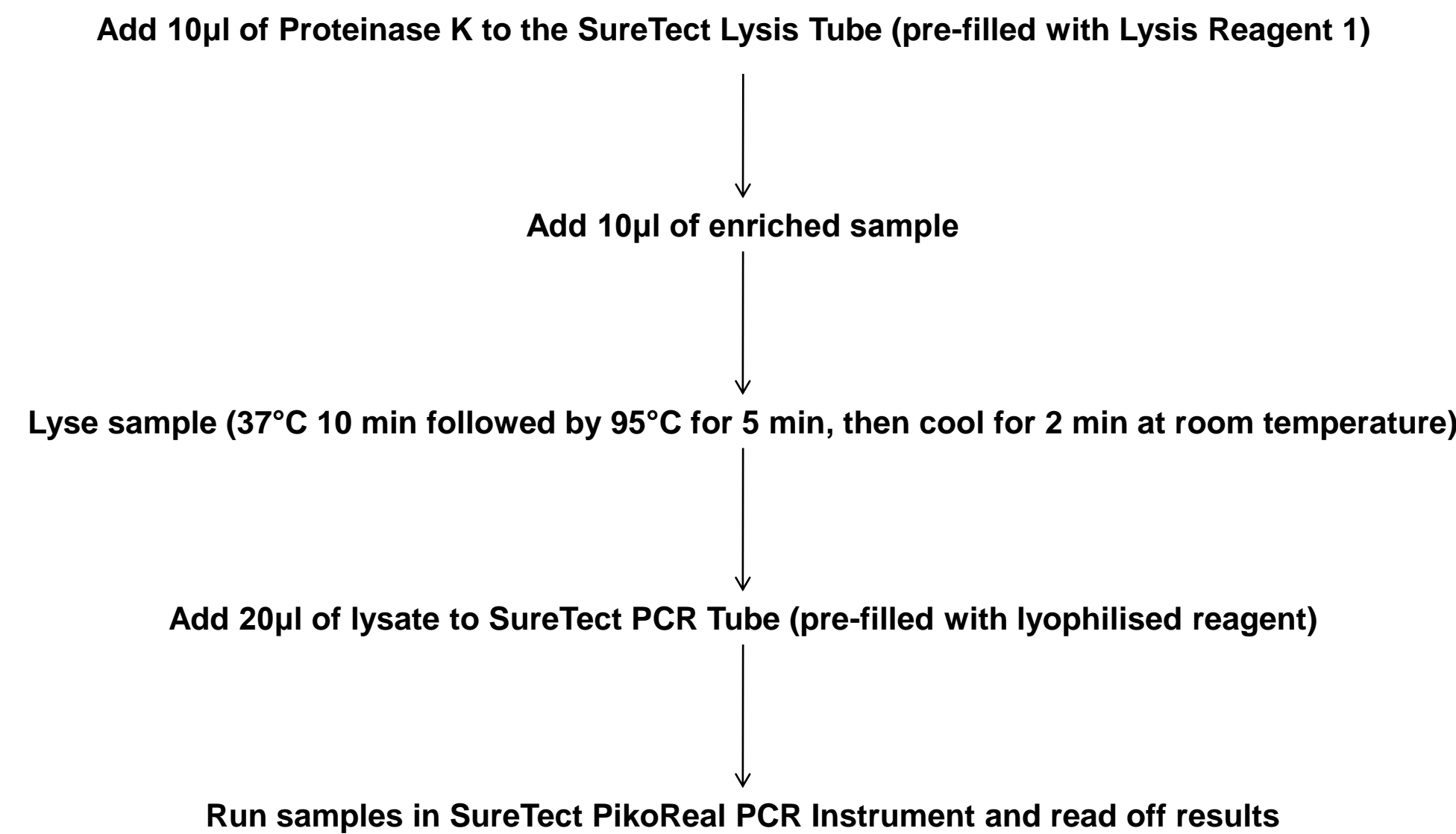
Samples of 375g dried chilli, onion and garlic powder were diluted 1:10 in TSB (chilli powder) or TSB + 5g/L K₂SO₃ (onion and garlic powders). Samples were spiked as shown in Figure 2. For chilli, 2 samples remained unspiked and for both onion and garlic powder, 3 samples remained unspiked. Homogeniser bags were hand mixed and left to stand for one hour before checking the pH and adjusting if necessary to 6.8 ± 0.2 before incubation for 20-22 h at 35°C. Following incubation, samples were analysed according to the SureTect Salmonella species PCR Assay (Figure 3). To confirm a positive result, the SureTect Salmonella species PCR Assay confirmation protocol was carried out by streaking onto Thermo Scientific™ Oxoid™ Brilliance™ Salmonella Agar. Any high background matrices, such as onion powder, required a secondary enrichment step in Thermo Scientific™ Oxoid™ Rappaport-Vassiliadis Soya Peptone (RVS) Broth prior to streaking onto Brilliance Salmonella Agar. Presumptive positive colonies obtained on Brilliance Salmonella Agar were confirmed using the Thermo Scientific™ Oxoid™ Salmonella Latex Test Kit. The FDA BAM method required secondary enrichment of the BPW primary enrichment in RVS Broth (42 ± 0.2°C for 24 ± 2 hours) and Thermo Scientific™ Oxoid™ Muller-Kauffmann Tetrathionate Novobiocin Broth (35 ± 2°C for 24 ± 2 hours). After incubation secondary broths were streaked onto Xylose-Lysine-Desoxycholate Agar, Thermo Scientific™ Oxoid™ Bismuth Sulphite Agar, Thermo Scientific™ Oxoid™ Hektoen Enteric Agar and Oxoid Brilliance Salmonella Agar. All culturally positive results (after 24 ± 2 hours at 35°C) were confirmed using the Salmonella Latex Test Kit.

Exclusivity isolates were tested with the SureTect Salmonella Species PCR Assay from turbid 18-24 h cultures in TSB (~1x10⁸ CFU/ml).

Figure 2: Spiking Protocol

Matrix	No. of spiked samples	Spike organism	Spike cfu / bag
Chilli Powder	5	<i>Salmonella</i> Montevideo	22.2
Onion Powder	11	<i>Salmonella</i> Oranienberg	12.8 – 112
Garlic Powder	8	<i>Salmonella</i> Montevideo	3.4 - 49

Figure 3: The SureTect Salmonella species PCR Assay Workflow



Results

All exclusivity isolates tested gave negative results with the SureTect Salmonella species PCR Assay (Figure 4).

Figure 4: Exclusivity Results

Organism name	No. tested	No. SureTect Salmonella Species PCR Assay positive results	No. SureTect Salmonella Species PCR Assay negative results
<i>Citrobacter freundii</i>	6	0	6
<i>Citrobacter youngae</i>	1	0	1
<i>Citrobacter koseri</i>	5	0	5
<i>Citrobacter amalonaticus</i>	1	0	1
<i>Enterobacter cloacae</i>	5	0	5
<i>Klebsiella pneumonia</i>	6	0	6

The SureTect Salmonella species PCR Assay results for chilli powder (Figure 5), onion Powder (Figure 6) and garlic powder (Figure 7) were comparable to the FDA BAM method results.

Figure 5: Comparison of the SureTect Salmonella species PCR Assay to the FDA BAM method for chilli powder

No. tested	Spike level cfu / sample	No. SureTect Salmonella species PCR Assay positive results	No. SureTect Salmonella species PCR Assay confirmed positive results	FDA BAM confirmed positive results
5	22.2	5	5	5
2	Unspiked	0	0	0

Figure 6: Comparison of the SureTect Salmonella species PCR Assay to the FDA BAM method for onion powder

No. tested	Spike level cfu / sample	No. SureTect Salmonella species PCR Assay positive results	No. SureTect Salmonella species PCR Assay confirmed positive results	FDA BAM confirmed positive results
5	12.8	0	0	0
2	29.8	2	2	2
2	57.8	2	2	2
2	112.0	2	2	2
3	Unspiked	0	0	0

Figure 7: Comparison of the SureTect Salmonella species PCR Assay to the FDA BAM method for garlic powder

No. tested	Spike level cfu / sample	No. SureTect Salmonella species PCR Assay positive results	No. SureTect Salmonella species PCR Assay confirmed positive results	FDA BAM confirmed positive results
5	3.4	0	0	0
1	13.0	1	1	1
1	25.2	1	1	1
1	49.0	1	1	1
3	Unspiked	0	0	0

Conclusion

All SureTect Salmonella species PCR Assay results were comparable to the FDA BAM results and all exclusivity strains and unspiked samples gave negative results with the SureTect Salmonella species PCR Assay. Due to the die-off of *Salmonella* on these matrices, a high spike level (13-30 CFU/sample) was required to achieve positive results with the SureTect Salmonella species PCR Assay and the FDA BAM method.

This study demonstrated that the SureTect Salmonella species PCR Assay can be used to detect *Salmonella* in 375 g samples of dried chilli, onion and garlic powder using a 1:10 dilution of the sample in TSB as described in the FDA BAM method.

References

1. FDA Bacteriological Analytical Manual Chapter 5 Salmonella May 2014 Version. Wallace H. Andrews, Andrew Jacobson, and Thomas Hammack.