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 Soy 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 within 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 5. 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 powder). 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 at room temperature for 30 min before enrichment of 16-20 hours in TSB + 5g/L K2SO3. 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 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 TSB + 5g/L K2SO3. Replicates of all samples (onion and garlic) were streaked onto Salmonella Agar. Presumptive positive colonies obtained on Brilliance Salmonella Agar were confirmed using the Thermo Scientific™ Oxoid™ Brilliance Salmonella Lysis Tube Kit. The FDA BAM method required secondary enrichment of 24 hours at 35°C. Following incubation, colonies were selected and streaked onto Xylose-Lysine-Deoxycholate Agar, Thermo Scientific™ Oxoid™ Brilliant Blue R Agar and Thermo Scientific™ Oxoid™ Brilliant Agar. All culturally positive isolates (after 24 hours at 35°C) were confirmed using the Salmonella Lysis Test Kit.

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

Figure 2: Spiking Protocol

Figure 3: The SureTect Salmonella species PCR Assay Workflow

Figure 4: Exclusivity Results

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

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

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

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 away of Salmonella on these matrices, a high spike level (130 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 spices. Further work on garlic and onion powder using a 1:10 dilution of the sample in TSB as described in the FDA BAM method.

References