Overview

Purpose: The aim of this study was to evaluate the performance of the Thermo Scientific™ SureTect™ Salmonella species PCR Assay (Thermo Fisher Scientific) for detection of Salmonella Tallahassee from dark chocolate (70% cocoa).

Methods: Samples of dark chocolate were combined with Thermo Scientific™ Oxoid™ Cocoa Sample Recovery (CSR) Broth (Thermo Fisher Scientific) in a 1:10 ratio and artificially contaminated with stressed Salmonella Tallahassee at three spike levels. Following incubation, enriched samples were tested using the SureTect Salmonella species PCR Assay. All positive and negative results were confirmed according to the manufacturer’s instructions.

Results: The SureTect Salmonella species PCR Assay showed high sensitivity, specificity and accuracy for detection of Salmonella Tallahassee from dark chocolate (70% cocoa).

Introduction

Salmonella is one of the most frequent causes of food poisoning and a major public health problem worldwide. Foods often contaminated with Salmonella include meat, poultry, milk and dairy products, eggs, seafood, and some fruits and vegetables. The detection of Salmonella in foods before they are consumed is vital for safeguarding public health, and essential for preserving the financial health and reputation of food businesses.

The SureTect Salmonella species PCR Assay is a real-time PCR test which is optimised for use in conjunction with the Thermo Scientific™ PikoReal™ Real-Time PCR Instrument (see figure 1) and the Thermo Scientific™ SureTect™ Software for the detection of Salmonella spp. in food and environmental samples.

Methods

Spiking of chocolate samples

Salmonella Tallahassee was cultured in Tryptone Soya Broth (TSB) at 37°C for 18-24 hrs. then heat stressed in a water-bath set at 55°C for 10 mins. Ten 25 g samples were individually spiked with low (1-5 CFU/25 g) (n=5), high (10-20 CFU/25 g) (n=3) concentrations of Salmonella Tallahassee. Two samples remained unspiked.

FIGURE 1. PikoReal Real-Time PCR Instrument and SureTect PCR Assays

SureTect Salmonella species PCR Assay

The ten 25 g chocolate samples were combined with 225 ml of CSR Broth and incubated for 20 hrs. at 37±1°C. Ten μl of each enriched chocolate sample was added to the prefilled SureTect Lysis Tubes (prepared by additionally adding Proteinase K Reagent) and the sample lysed according to the SureTect lysis protocol (37°C for 10 mins followed by 95°C for 5 mins). Once lysed, 20 μl of the lysate was added to the SureTect PCR Tubes (contain lyophilised PCR reagents). Then the SureTect PCR Tubes were loaded into the SureTect PikoReal Real-Time PCR instrument and the PCR run was started. After approximately 1 hr and 20 mins, the SureTect Salmonella species PCR Assay results were automatically interpreted as ‘positive’ or ‘negative’ by the SureTect Software.

Confirmation of results

All results from the SureTect System were confirmed culturally using the SureTect confirmation method as stated in the IFU. Enriched samples were streaked onto Thermo Scientific™ Oxoid™ Brillance™ Salmonella Agar and incubated at 36±1°C for 24 hrs. Presumptive positive purple colonies observed on Brillance Salmonella Agar were confirmed using Thermo Scientific™ Oxoid™ Salmonella Latex Kit and Thermo Scientific™ Remel™ Salmonella O Polyvalent agglutinating sera.

Data analysis

The data obtained was used to calculate the sensitivity, specificity and accuracy for the SureTect Salmonella species PCR Assay.

TABLE 1. Performance of SureTect Salmonella species PCR Assay

<table>
<thead>
<tr>
<th>Performance measure</th>
<th>SureTect Salmonella species PCR Assay</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity (low spike)</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity (high spike)</td>
<td>100%</td>
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<tr>
<td>Sensitivity (overall)</td>
<td>100%</td>
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<tr>
<td>Specificity</td>
<td>100%</td>
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<tr>
<td>Accuracy</td>
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Conclusion

SureTect Salmonella species PCR Assay showed 100% sensitivity, specificity and accuracy in detecting Salmonella Tallahassee from dark chocolate (70% cocoa) at both low and high-level spike levels.

References