Detection of Salmonellae from Production Environment Samples by the Thermo Scientific SureTect Salmonella Species PCR Assay

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Overview

Purpose: To compare performance of the Thermo Scientific™ SureTect™ Salmonella species PCR Assay with the ISO reference method for detection of salmonellae from production environment samples.

Methods: The SureTect method was compared to the reference method detailed in ISO 6579:2002.

Results: The SureTect Salmonella species PCR Assay reliably detected the presence of *Salmonella* in production environment samples.

Introduction

Food safety and quality legislation[2] in many developed countries is based on HACCP, combined with British Retail Consortium standards[3] and company protocols which require monitoring of pathogen and/or indicator organisms within food production environments to ensure compliance with hygiene standards.

The SureTect Salmonella species PCR Assay (PT0100A) is a real-time PCR test for the detection of *Salmonella* from foods and food production environment samples, which combines pre-dispensed lysis reagent and lyophilised, tableted PCR reagents to simplify and improve assay handling, along with dedicated software to run the assays and automatically interpret results. This study was conducted to evaluate performance of the assay in comparison to ISO 6579:2002.

Methods

Sample Preparation

Seventy-four samples consisting of 30 positive and 44 negative samples originating from food production environments were analysed during this study which formed part of an independent laboratory study. Samples consisted of: siphon, process and rinse waters, surface sampling wipes and dust. Three positive samples were naturally contaminated, while the remainder of positive samples were spiked with dilutions of stressed isolates of the following serotypes of *Salmonella*: Amsterdam, Typhimurium, Agona, Blockley and Indiana at 1.0-11.4 CFU/sample, at the point of sample homogenisation with prepared Buffered Peptone Water (ISO). Stressing of isolates was accomplished by storage at 4°C for 15 days to 2 months at either pH 4 or 7.

SureTect Assay Method

Twenty-five g or ml of solid or liquid samples or complete surface wipes were added to 225 ml of room temperature BPW (ISO), for swabs the sample was added to 10 ml BPW (ISO) and for sponges the complete sampling surface of the swab was added to 100 ml of BPW (ISO). All samples were incubated at 37 ±1°C for 20 h.

Following enrichment, 10 µl of each sample was processed and analysed as detailed in the SureTect assay lysis protocol. After lysis, 20µl of the lysate was added to the SureTect PCR Tubes, (containing lyophilised PCR reagents) before running on the Thermo Scientific™ PikoReal™ Real-Time PCR Instrument.

Assay results were automatically interpreted as "positive" or "negative" by the SureTect Software. All results were confirmed by the SureTect confirmation protocol (sub-culture in RVS Broth followed by direct plating onto Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar. Presumptive positive colonies were confirmed using either the Thermo Scientific™ Oxoid™ Salmonella latex kit (DR1108A) or Thermo Scientific™ Oxoid™ Microbact™ GNB 24E biochemical kit.

ISO Reference Method

The reference method detailed in ISO 6579:2002 was followed, using *Brilliance* Salmonella Agar as the second plating medium. Confirmations were performed according to the reference method.

Results

Twenty-eight of the 30 positive samples analysed in this study were correctly identified as positive by the SureTect Salmonella assay, including from samples spiked at very low levels. Positive PCR results were all correctly confirmed by culture and either of the two confirmation kit options (latex agglutination or biochemical micro-gallery kits). Two samples positive with the reference method were not positive by PCR. Both of these samples were confirmed using the SureTect confirmation protocol, which was conducted on all samples in this study. One of these samples was naturally contaminated, therefore secondary enrichment (during the reference method) would have improved the chance of recovering low levels of stressed *Salmonella*, which are often present in this sample type.

FIGURE 1. Results for Positive Samples

Sample Type	SureTect PCR Positive	SureTect Confirmation Method Positive	ISO Method Positive
Siphon water	2	2	2
Rinse water	2	2	2
Process water	7	8	8
Wipes/Dust	17	18	18

Conclusion

The SureTect Salmonella species PCR Assay was shown to be a suitable alternative rapid method for the identification of *Salmonella* from food production environment samples compared to the reference method detailed in ISO 6579:2002.

References

1. Microbiology of Food and Animal Feeding stuffs-Horizontal Method for the Detection *Salmonella* spp. ISO 6579:2002.
2. Regulation (EC) 852/2004, Official Journal of the European Union.
3. British Retail Consortium; Global Standard Food Safety Issue 7: 2015.

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