# Detection of salmonella in chocolate matrices using *mericon*® assays and QIAsymphony® RGQ

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In this application note, we describe a food safety testing solution used to reliably identify salmonella in chocolate products, which are particularly challenging food matrices. The workflow involves automated sample preparation and assay setup on the QIAsymphony and pathogen detection via real-time PCR using the *mericon* Salmonella spp assay on the Rotor-Gene Q®.

## Introduction

Food production requires highly sensitive pathogen detection. Real-time PCR meets this need. It is highly specific and time-effective. While it takes several days to obtain microbiological product approval with traditional culturing methods, it takes less than 24 h from sample preparation to result with real-time PCR.

The complexity of food matrices can pose significant challenges to the adoption of such molecular techniques. Developments in sample preparation and assay technologies are overcoming these challenges.

One of the most economically significant food-borne pathogens is the Gram-negative bacterium salmonella, which has been found in a wide variety of foodstuffs, including poultry, eggs, meat, and peanut butter, and recently also in chocolate products. Chocolate is a particularly demanding food matrix, as it has high lipid contents and contains substances such as polyphenols, which can interfere with downstream PCR reactions if not removed during the purification process.

In this study, *Salmonella* spp DNA was purified from enrichment cultures of several chocolate-containing food matrices, and detection was performed with the *mericon* Salmonella spp assay. The workflow was completely automated with the modular QIAsymphony RGQ (Figure 1), comprising the QIAsymphony SP/AS for sample purification and assay setup, and the Rotor-Gene Q thermal cycler for real-time PCR.

**Figure 1. The QIAsymphony RGQ, comprising the QIAsymphony SP/AS instruments and the Rotor-Gene Q.**

## Materials and methods

### Enrichment cultures

The food matrices used were milk chocolate, chocolate with liquid coffee filling, and hazelnut spread. Two enrichment culture protocols (small: 25 g food sample input; and large: 750 g food sample input) were performed. All of the mixtures were spiked with ~10–20 cfu *Salmonella* spp and incubated for 20 h at 37°C. Sample aliquots of 1 ml were removed for sample purification.

### Sample purification

The QIAsymphony SP/AS is a modular system for the extraction of DNA from up to 96 samples and subsequent assay setup. Enrichment culture samples were subjected to thermal pathogen lysis at 90°C, followed by capture of pathogen DNA using QIAsymphony bead technology. Optimized washing steps removed inhibitors prior to the elution of pure pathogen DNA.

### *Salmonella* spp detection

*Salmonella* spp DNA was detected using the *mericon* Salmonella spp kit. This assay consists of highly specific primers and probes for *Salmonella* spp, and contains an internal inhibition control and a Multiplex PCR Master Mix. Assay setup was performed on the QIAsymphony AS and detection on the Rotor Gene Q, which is a precise and versatile real-time PCR cycler with a unique centrifugal rotary design. All of the *mericon* food assays are optimized for use with the Rotor-Gene Q, but are also compatible with all other real-time PCR cyclers.

## Results

### Sensitivity and robustness of the *mericon* assay

The detection sensitivity of the *mericon* Salmonella spp Kit was tested using purified *Salmonella* spp DNA on the Rotor-Gene Q. Titration was carried out in 1:10 dilutions from 10,000 to 10 copies per PCR reaction (Figure 2).

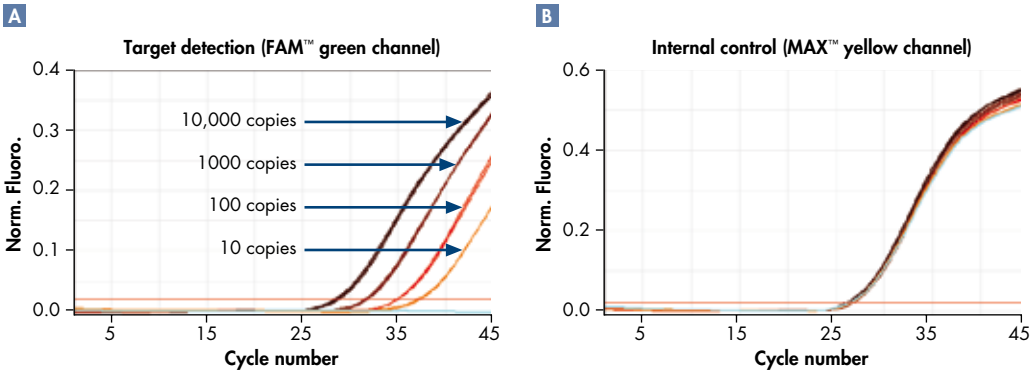


**Figure 2. Highly sensitive and robust assay.** A Template DNA amplified in duplicate, detection in the green channel ($R^2$ = 0.993). B Internal Control, MAX™-labeled, detection in the yellow channel.

Master Mix resistance to the presence of inhibitors was assessed (Figure 3). No changes in threshold value were seen, while the addition of inhibitors to an assay from another supplier resulted in increases of 3–10 $C_T$, indicating that the Master Mix is more resistant to the presence of inhibitors.

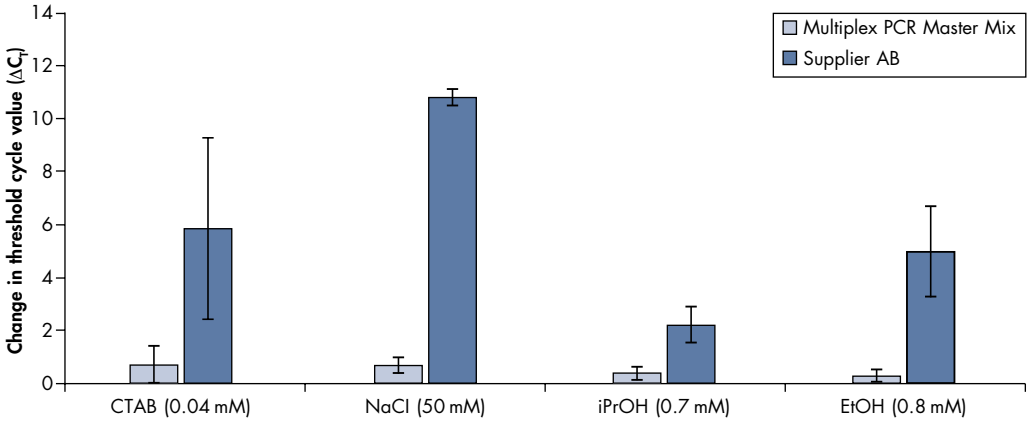


**Figure 3. Heightened resistance to inhibitors.** A plasmid carrying an artificial DNA sequence (DNA model TaqMan® system, $10^8$ copies) was amplified in reactions spiked with inhibitors potentially carried over from sample preparations. The change in threshold cycles ($C_T$) of each inhibitor compared to that of a water negative control is shown.

### Salmonella detection in food matrices containing chocolate on the QIAsymphony RGQ

Automated pathogen DNA extraction and detection was performed using the QIAsymphony RGQ. A culture of *Salmonella* spp in a matrix of either chocolate with liquid coffee filling or milk chocolate was prepared and processed in different dilutions. Efficient DNA extraction is reflected in the consistent PCR titration. The PCR results of the culture dilutions as well as the internal control show no assay inhibition (Figure 4 and 5). Chocolate hazelnut spread was also tested under the same conditions and yielded the same results (data not shown).

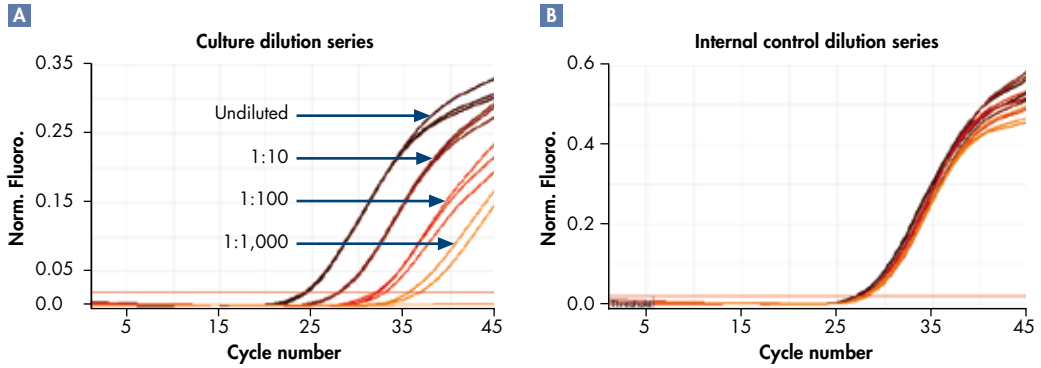


**Figure 4. No inhibition with difficult food matrices.** 750 g chocolate with coffee filling was homogenized in 7.5 liters buffered peptone water, inoculated with ~10–20 cfu *Salmonella* spp and enriched for 20 h at 37°C. Cultures were diluted as indicated and *Salmonella* was detected using the *mericon* Salmonella spp. Kit. A Culture dilution curves. B Internal Control curves.

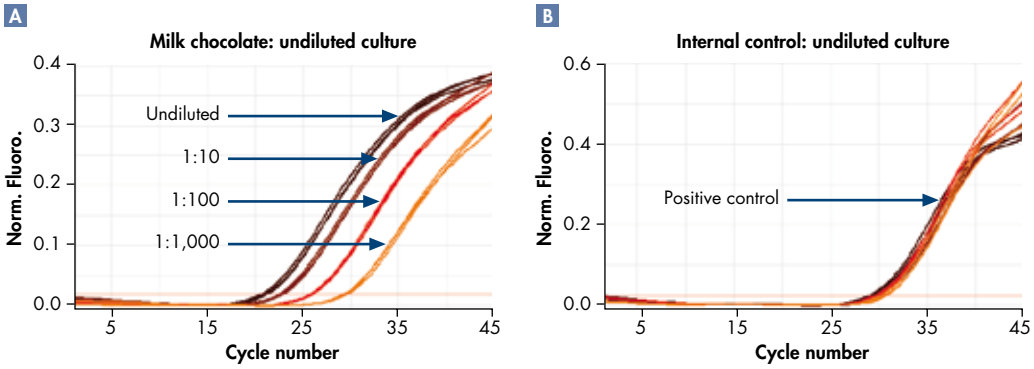


**Figure 5. Efficient detection of *Salmonella* in chocolate.** 25 g milk chocolate was homogenized in 225 ml buffered peptone water, inoculated with ~10 cfu *Salmonella* spp and enriched for 20 h at 37°C.

## Conclusion

- The *mericon* Salmonella spp Kit in combination with the QIAsymphony RGQ provides an optimized workflow for efficient and reliable routine sample processing of challenging food matrices, such as milk chocolate, chocolate with liquid coffee filling, and chocolate hazelnut spread.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| *mericon* Salmonella spp Kit (96)* | For 96 reactions: PCR Assay Salmonella spp, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect® Nucleic Acid Dilution Buffer, RNase-free water | 290015 |
| QIAsymphony RGQ, System | QIAsymphony SP, QIAsymphony AS, Rotor-Gene Q 5plex HRM; includes required accessories and consumables, installation, and training; includes 1-year warranty on parts and labor | 9001850 |

* Smaller kit size also available

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

**Discover the *mericon* food testing portfolio and learn how real-time PCR can revolutionize your testing program. Visit www.qiagen.com/foodsafety.**



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