Novel solutions for molecular food safety testing

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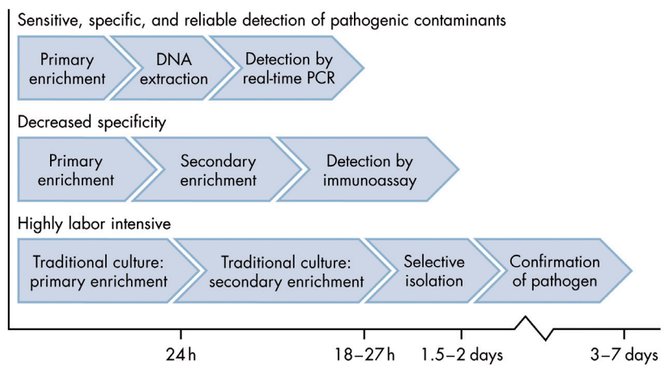
Introduction

Food safety concerns have steadily increased in recent years and are now one of the most important challenges for food authorities. There is a requirement for highly sensitive pathogen detection and most food testing laboratories rely on time-consuming culturing methods, with associated high storage and production costs.

QIAGEN has recently introduced the *mericon*™ Food Safety Testing product line, covering reagent kits and instruments for all major food applications – from DNA extraction to highly specific real-time PCR detection.

The data shown here illustrate the sensitivity of the new *mericon* PCR assays, as well as tolerance of the Multiplex PCR Master Mix to PCR inhibitors. Representative pathogen DNA extractions from food enrichment cultures and detection with the *mericon* assays are shown for *Salmonella enterica* and *Listeria monocytogenes*. Efficiency of 2 new DNA extraction systems for Gram-positive and -negative bacteria are compared for cultured samples with and without food matrices.

We also present the QIAsymphony® Rotor-Gene® Q, a fully automated system for DNAextraction directly from enrichment cultures, followed by automated reaction setup, and detection on the Rotor-Gene Q real-time PCR instrument. The protocol setup and results of QIAsymphony extraction detection runs for food enrichment cultures are described.



Comparison of time-to-result of different methods for pathogen detection.

The QIAGEN food safety testing pathogen workflow

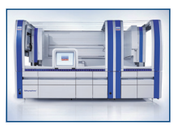
Sample preparation → Real-time PCR assays → Detection

***mericon* DNA Bacteria Kit**
- Thermal lysis for Gram-negative bacteria.

***mericon* DNA Bacteria Plus Kit**
- Mechanical lysis for Gram-positive and Gram-negative bacteria.

The TissueLyser LT, for simulaneous mechanical disruption of 12 samples.

The QIAsymphony Rotor-Gene Q system
- Fully automated instrument for pathogen DNA or RNA extraction, assay setup, and detection.



The QIAsymphony Rotor-Gene Q.

***mericon* PCR detection kits**
- 11 real-time PCR assays for all major food-borne pathogens.

The *mericon* portfolio includes:
- *Salmonella* spp.
- *L. monocytogenes*
- *Listeria* spp.
- *C. sakazakii*
- *Campylobacter* spp.
- *C. jejuni, laridis, coli*
- *E. coli* VTEC stx 1/2
- *S. aureus*
- *Y. enterocolitica*
- *Shigella* spp.
- *Legionella* spp.
- *L. pneumophila*

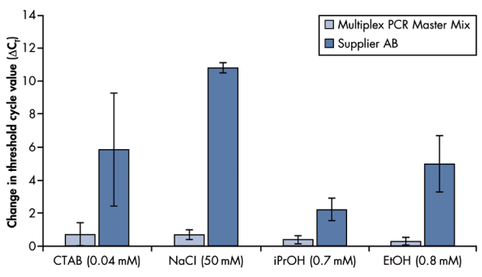
Rotor-Gene Q real-time PCR cycler
- All *mericon* assays are optimized for use with the Rotor-Gene Q, but are also compatible with other real-time (block) cyclers.

The Rotor-Gene Q is a precise and versatile real-time PCR cycler with a unique centrifugal rotary design.

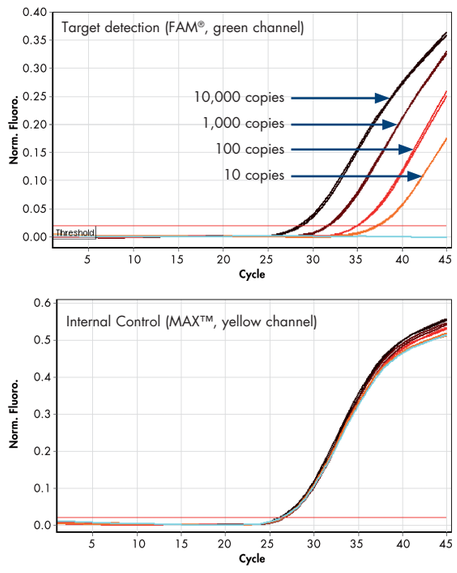
***mericon* assays.**

Real-time PCR pathogen detection in complex food matrices: *mericon* Assay systems

- Assay portfolio includes all major food-borne pathogens.
- Highly sensitive target detection down to 10 copies.
- Internal Control to indicate possible PCR inhibition.
- PCR Multiplex Master Mix highly tolerant to PCR inhibitors.
- Combine several assays in one run; all *mericon* PCR assays use the same cycling conditions.



Comparison of resistance to residual PCR inhibitors of the *mericon* Multiplex PCR Master Mix and a master mix provided by supplier AB. 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 of each inhibitor compared to that of a water negative control is shown.



The *mericon* Salmonella spp Kit detection sensitivity with purified *Salmonella* DNA on the Rotor-Gene Q. Titration was carried out in 1:10 dilutions from 10,000 to 10 copies per PCR reaction. Above: Template DNA amplified in duplicates, detection in the green channel (R^2=0.993). Below: Internal Control, MAX-labeled, detection in the yellow channel.

Fully automated pathogen DNA extraction and detection: QIAsymphony Rotor-Gene Q

QIAsymphony Rotor-Gene Q System

Sample preparation
- Loading of untreated enrichment culture
- Thermal lysis at 90°C for 5 min
- DNA sequestration via magnetic, silica-coated beads
- Automated transfer of elution plate to assay setup module

Processing of 48 samples:
1 ml protocol → 140 min
200 µl protocol → 96 min

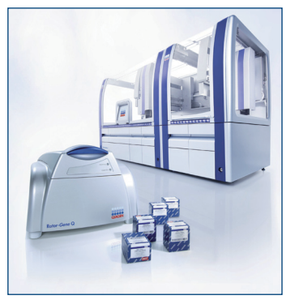
Assay setup
- Automated data transfer for continuous sample tracking
- Highly accurate pipetting into Rotor-Gene Q ring*

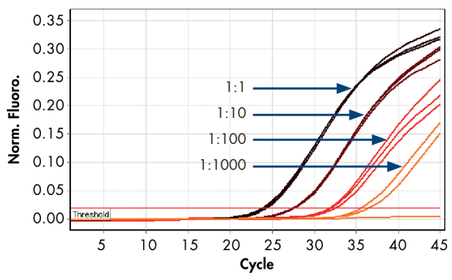
Protocol time: 25 min

Real-time PCR detection
- PCR target detection via integrated Rotor-Gene Q instrument

Protocol time: 74 min

* Or other applied PCR cycler formats.

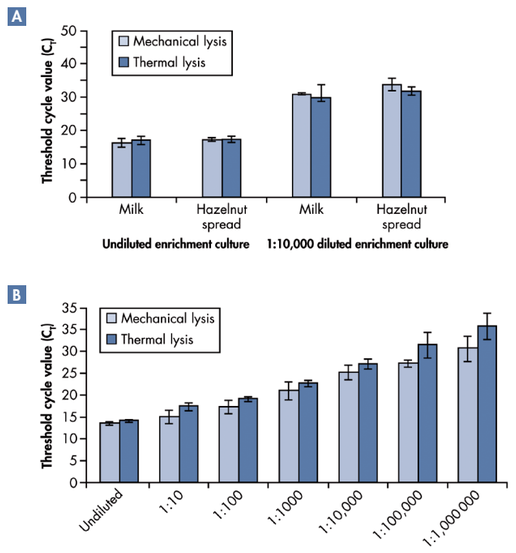




Automated pathogen DNA extraction and detection with the QIAsymphony Rotor-Gene Q. 750 g chocolate with coffee filling was homogenized in 6.75 l buffered peptone water and inoculated with ~10 cfu *Salmonella*. Detection was performing using the *mericon* Salmonella spp Kit.

Time-efficient manual pathogen DNA extraction and detection: *mericon* DNA Bacteria Kits

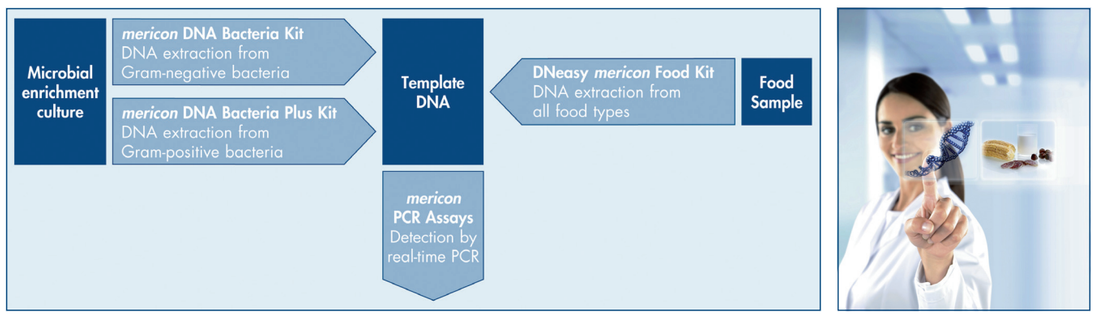
- QIAGEN introduces 2 new manual systems for pathogen DNA extraction from enrichment cultures.
- The *mericon* DNA Bacteria Plus Kit is based on a mechanical lysis using glass beads. The method was developed for Gram-positive or difficult to lyse bacteria, but is universally applicable.
- The *mericon* DNA Bacteria Kit is based on a thermal pathogen lysis for Gram-negative or easy to lyse bacteria.
- Pathogen DNA extraction is reduced to a minimum of simple, time-efficient steps; 30 samples can be processed in ~30 minutes.



Comparison of DNA extraction efficiency for Gram-negative and Gram-positive bacteria. A 25 g of milk or hazelnut spread was homogenized in 225 ml buffered peptone, inoculation with ~10 cfu *Salmonella* and enriched for 20 h at 37°C. Undiluted and diluted enrichment culture samples were processed using both *mericon* DNA Bacteria Kits and tested with the *mericon* Salmonella spp Kit on the Rotor-Gene Q. Overall, the efficiency of DNA extraction from Gram-negative bacteria is similar with both preparation protocols. B Serial dilutions of enrichment cultures of *S. enterica* (buffered peptone water) and *L. monocytogenes* (1/2 Fraser medium) were were processed using both *mericon* DNA Bacteria Kits. For *L. monocytogenes* (Gram-positive), mechanical lysis delivered a more effective extraction, most evident at low bacterial concentrations where the average C_T was 4.9 cycles lower with mechanical lysis.

Conclusions

- QIAGEN introduces a comprehensive product portfolio for all major applications in molecular food safety testing.
- The new *mericon* products cover reagent kits and instruments from DNA extraction to real-time PCR detection.
- The portfolio contains sample preparation and detection solutions for all major food pathogens, as well as complete solutions for GMO, animal, plant, and allergen detection.



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