November 2013

Product Insert for the AOAC-RI PTM-certified and NF VALIDATION certified *mericon*® Automated and Manual Salmonella Detection Workflows

QIA 36/01 – 02/13
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
www.afnor-validation.com

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

DNA extraction — automated workflow

QIAsymphony® *mericon* Bacteria Kit	(360)
Catalog no.	**931156**
Number of reactions	**360**
Reagent Cartridge*	2
Piercing Lid	2
TopElute Fluid	60 ml
Reuse Seal Set†	2
Product Insert	1
Quick-Start Protocol	1

* Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† A Reuse Seal Set contains 8 Reuse Seal Strips.

DNA extraction — manual workflow

	***mericon* DNA Bacteria Kit (100)**	***mericon* DNA Bacteria Plus Kit (50)**
Catalog no.	**69525**	**69534**
Number of preps	**100**	**50**
Fast Lysis Buffer	1 x 25 ml	2 x 25 ml
Pathogen Lysis Tubes L	–	5 x 10
Product Insert	1	1
Quick-Start Protocol	1	1

Real-time PCR — automated and manual workflows

***mericon* Salmonella spp.**		**(24)**	**(96)**
Number of reactions		**24**	**96**
Yellow	*mericon* Assay*	2 x 12 reactions	1 x 96 reactions
Red	Positive Control DNA	20 reactions	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	1.5 ml
	RNase-Free Water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 µl
	50x ROX Dye Solution	45 µl	210 µl
Product Insert		1	1
Quick-Start Protocol		1	1

* Contains target-specific primers and probes, as well as the internal control (IC)

† Contains HotStarTaq® *Plus* DNA Polymerase, dedicated multiplex real-time PCR buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP).

Storage and Shelf Life

The QIAsymphony *mericon* Bacteria Kit should be stored at room temperature (15–25°C). Do not store the reagent cartridges at temperatures below 15°C. When stored properly, the kit is stable until the expiration date stated on the kit box. Partially used reagent cartridges can be stored for a maximum of 1 month. If a reagent cartridge is partially used, reseal all troughs with the provided Reuse Seal Strips. To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 48 hours (including run times) at ambient temperature. Fast Lysis Buffer should be stored dry at room temperature (15–25°C). Under these conditions, the kit remains stable for 2 years.

The *mericon* Salmonella spp. Assay is shipped on dry ice. The Multiplex PCR Master Mix should be stored immediately at –20°C upon receipt. All remaining kit components not reconstituted should be stored at 2–8°C and protected from light. Stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope. Reconstituted reagents of *mericon* Pathogen Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at 2–8°C for short-term storage (1 month) or at –20°C for long-term storage.

Intended Use

Products for the automated and manual *mericon* Pathogen Detection workflows are intended for molecular biology applications in food, animal feed, water, and pharmaceutical product testing. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to regional guidelines that have been developed for working with pathogens and recombinant DNA.

Intended User

The automated and manual *mericon Salmonella* spp. detection workflows are designed to be used by qualified users in microbiology laboratories for the determination of the presence or absence of *Salmonella* spp. in food products.

Applicability

AOAC-RI PTM: The *mericon* Salmonella spp. detection workflows have been evaluated in an independent laboratory for use with the following food matrices: Ground beef (30% fat), spinach, peanut butter, non-fat dry milk, chicken carcass rinses, milk chocolate, whole milk, and shell eggs. The protocol includes preparation of an enrichment culture, followed by a manual or automated purification of *Salmonella* spp. DNA, and real-time PCR assay for presence or absence of pathogen using the *mericon* Salmonella spp. detection assay on the Rotor-Gene® Q.

NF VALIDATION: The *mericon* Salmonella spp. detection workflows have been evaluated in an independent laboratory for use with all human food products, animal feedstuffs and environmental samples (excluding primary production stage environment) The protocol includes preparation of an enrichment culture, followed by a manual or automated purification of *Salmonella* spp. DNA, and real-time PCR assay for presence or absence of pathogen using the *mericon* Salmonella spp. detection assay on the Rotor-Gene® Q.

Environmental Factors

To allow for optimal real-time PCR detection quality using the Rotor-Gene Q, the instrument should be installed in a temperature-controlled, draft-free laboratory. Temperature should not be below 68°F (20°C) and should not fluctuate during the performance of the PCR assay. If the ambient temperature

is below 68°F (20°C), it is recommended to preheat the Rotor-Gene Q at 95°C for 20 minutes before the run.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

For safety information regarding the instruments, see the relevant instrument user manual.

Discard sample and assay waste according to your local safety regulations.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Technical Assistance

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Please also refer to the handbooks for the kits and user manuals for the instruments for comprehensive Troubleshooting Guides. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

General Precautions for Real-Time PCR Assays

The *Salmonella* spp. pathogen detection assay involves DNA detection by PCR. Care must be taken to avoid contamination of the PCR reactions.

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

General physical and chemical precautions

- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Use a separate set of pipets for the PCR master mix and the DNA samples. Use of pipet tips with hydrophobic filters is strongly recommended.
- Use gloves and protective laboratory wear. Do not touch any PCR equipment and supplies (e.g., rotors, loading blocks, tubes, pipets) without wearing gloves.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.
- All materials and media possibly containing the tested pathogen should be autoclaved for 20 min at 120°C prior to disposal.

Equipment and Reagents to Be Supplied by User

Automated Workflow

For the preparation of salmonella food enrichment cultures

- Lab paddle blender (e.g., Stomacher® 400 Circulator, Seward)*
- Filter homogenizer bags (e.g., VWR®, cat. no. 129-9874)
- Balance*

For sample preparation

- QIAsymphony SP instrument (cat. no. 9001297)*
- QIAsymphony *mericon* Bacteria Kit (cat. no. 931156)

Accessories and adapters for the QIAsymphony SP

- Reagent Cartridge Holder (2) (cat. no. 997008)
- Insert, 2.0ml v2, sample carrier. (24), Qsym (cat. no. 9242083)
- Cooling Adapter, EMT, v2, Qsym (cat. no. 9020730)

Consumables for the QIAsymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Microtubes 2 ml, PP, without lids (Sarstedt®, cat. no. 72.608)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Elution Microtubes CL with cap strips (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)

For assay set-up

- QIAsymphony AS instrument (cat. no. 9001301)*
- *mericon* Salmonella spp. Kit (cat. nos. 290013 or 290015)

Accessories and adapters for the QIAsymphony AS

- Cooling Adapter, Reagent Holder 1, Qsym (cat. no. 9018090)

* Ensure that all instruments have been checked and calibrated according to the manufacturer's recommendations

For use with the Rotor-Gene Q 72 Rotor-Disc® (cat. no. 9018899)

- Adapter 2 x Rotor-Disc, Qsym (cat. no. 9242204)
- Rotor-Disc 72 Loading Block (cat. no. 9018910)
- Rotor-Disc 72 (cat. no. 981303 [240]/981301 [24])
- Rotor-Disc Heat Sealing Film (cat. no. 981604 [600]/981601 [60])
- Rotor-Disc Heat Sealer (cat. no. 9018898 [110 V]; cat. no. 9019725 [230 V])
- Rotor-Disc 72 Locking Ring (cat. no. 9018900)

Consumables for the QIAsymphony AS

- Filter-Tips, 200 μl (cat. no. 990332)
- Filter-Tips, 50 μl (cat. no. 997120)
- Micro tubes 2 ml, PP, without lids (Sarstedt, cat. no. 72.608)
- Tip disposal bags (cat. no. 9013395)

Manual Workflow

For the preparation of salmonella food enrichment cultures

- Lab paddle blender (e.g., Stomacher 400 Circulator, Seward)*
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)
- Balance*

For sample preparation

- *mericon* DNA Bacteria Kit (cat. no. 69525)
- Vortexer
- SafeSeal Micro tubes 2 ml (Sarstedt, cat. no. 72.695) or microcentrifuge tubes with screw caps (2 ml)
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes
- Thermomixer* or heating block* suitable for 1.5 or 2 ml tubes and capable of attaining a temperature of 100°C. Alternatively, a water bath may be used.
- Pipets and pipet tips

* Ensure that all instruments have been checked and calibrated according to the manufacturer's recommendations.

For assay set-up

- Pipets and filter pipet tips

For use with the Rotor-Gene Q 72-Well Rotor (cat. no. 9018903)

- Loading Block, RG Strip Tubes 72, Qsym (cat. no. 9018092)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Locking Ring 72-Well Rotor (cat. no. 9018904)

Real-Time PCR

- Rotor-Gene Q instrument (cat. no. 9001550)*
- Rotor-Gene Q software version 2.02

* Ensure that all instruments have been checked and calibrated according to the manufacturer's recommendations.

Specifications of the AOAC-RI PTM-Certified salmonella Detection Workflow

The automated and manual salmonella workflows have received AOAC-RI PTM certified status (certificate 071204). The specifications for these workflows and the limit of detection can be found in Tables 1 and 2. For PCR assay setup, elution volumes of the automated workflow, and eluate dilutions of the manual workflow, are given in Table 3.

Table 1. Overview of the specifications

AOAC-RI PTM-certified specification	Details
Target	*Salmonella* spp.
DNA extraction Kit	**Automated workflow**: QIAsymphony *mericon* Bacteria Kit **Manual workflow**: *mericon* DNA Bacteria Kit
Real-time PCR assay	*mericon* Salmonella spp. Kit
Enrichment broth	Buffered peptone water Chocolate: Buffered peptone water with skim milk + brilliant green
Enrichment temperature	37°C
Enrichment time	18 ± 2 hours
Homogenizer bag	Filter bag
Sample matrices	Ground beef (30% fat) Chicken carcass rinses Creamy, non-organic peanut butter Fresh spinach Pasteurized whole milk Instant non-fat dry milk Milk chocolate Shell eggs

Table 2. Limit of detection

Workflow segment	Limit of detection
Overall automated and manual workflow	10^3 cfu/ml
mericon Salmonella spp. kit	10 copies/reaction

Table 3. Sample volumes for *mericon* assay setup (AOAC-RI PTM)

Matrix	Manual workflow eluate dilution	Automated workflow elution volume
Peanut butter	Undiluted	200 µl
Spinach	Undiluted	200 µl
Eggs	1:50	200 µl
Non-fat dry milk	Undiluted	200 µl
Whole milk	Undiluted	200 µl
Chicken carcass rinses	1:50	400 µl
Chocolate (milk)	1:50	400 µl
Ground beef (30% fat)	1:50	400 µl

Specifications of the AFNOR-Certified salmonella Detection Workflow

QIAGEN *mericon Salmonella spp.* automated and manual workflows are certified NF VALIDATION under the reference QIA 36/01 – 02/13 for the detection of *Salmonella* spp. in all human food products, animal feedstuffs and environmental samples (excluding primary production stage environment). The end of validity of the certification can be found by reference to the NF VALIDATION certificate available on www.afnor-validation.com or upon request to QIAGEN GmbH.

In the context of NF VALIDATION, all samples identified as positive by the QIAGEN *mericon* Salmonella spp. pathogen detection assay must be confirmed from the enriched BPW broth implementing the conventional tests described in the methods standardized by CEN of ISO from colonies (including the purification step). In the event of discordant results (presumptive positive with the alternative method, but non-confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

The QIAGEN method complies with

- Good Laboratory Practice (refer to EN ISO 7218 standard).
- In the context of NF VALIDATION, test portions weighing more than 25 g have not been tested.
- For the preparation of initial suspensions, follow the instruction of EN ISO 6579 and of EN ISO 6887 standards.

The specifications for these workflows can be found in Tables 4a. Detection limits are found in Table 4b. For PCR assay setup, elution volumes of the automated workflow, and eluate dilutions of the manual workflow, are given in Table 4c.

Table 4a. Overview of the specifications

NF VALIDATION specification	Details
Target	*Salmonella* spp.
DNA extraction Kit	**Automated workflow**: QIAsymphony *mericon* Bacteria Kit **Manual workflow**: *mericon* DNA Bacteria Kit
Real-time PCR assay	*mericon* Salmonella spp. Kit
Enrichment broth	Buffered peptone water
Enrichment temperature	37 ± 1°C
Enrichment time	18 ± 2 hours
Homogenizer bag	Filter bag
Sample matrices	All human food products Feedstuffs Environmental samples (excluding primary production stage environment)

Table 4b. Limit of detection

Workflow segment	Limit of detection
Both automated and manual workflow	Between 0.2 - 2.2 cells/25 g
mericon Salmonella spp. kit	10 copies/reaction

Table 4c. Sample dilutions for PCR Assay

NF VALIDATION specification	Details
Manual workflow	1:10 dilution of DNA eluate
Automated workflow	**QIAsymphony** 200 µl elution volume

Confirmation of Positive Results:

All positive *mericon* Salmonella results are presumptive positives and require confirmation by a reference method. In the scope of NF VALIDATION, any presumptive positive results should be confirmed by the standard NF EN ISO 6579 (2002) method for the determination of *Salmonella spp* in foods. In this case, an aliquot of the overnight buffered peptone water enrichment broth should be tested.

PRE-ENRICHMENT

X g (ml) + 9X ml buffered peptone water

Incubation for (18± 2) h at (37±1) °C

SELECTIVE ENRICHMENT

0.1 ml + 10 ml RVS	1 ml + 10 ml MKTTn
Incubation for (24±3) h at (41.5±1) °C	Incubation for (24±3) h at (37±1) °C

SELECTIVE AGAR ISOLATION

Each selective broth isolated on selective agar media (XLD and another selective agar)

Incubation for (24±3) h at (37±1) °C

CONFIRMATION

At least one typical colony per Petri dish and 4 others if the first is negative

Biochemical and serological confirmation after purification on nutritive agar.

Alternatively, any other method that has received NF VALIDATION can be utilized as a confirmatory method. The validated detection protocol of this second method must be followed entirely and is started from the buffered peptone water enrichment step that is common to both methods.

Protocol: Preparation of a salmonella Enrichment Culture

Procedure

1. Add 25 g of the potentially contaminated food sample to a filter homogenizer bag and add 225 ml buffered peptone water.
2. Homogenize the food sample using a lab paddle blender at 230 rpm for 1.5 min. Then, seal the homogenizer bag and incubate the homogenate for 18 ± 2 hours at 37°C.
3. Milk chocolate requires a unique enrichment scheme. Add a 25 g sample to 225 ml buffered peptone water with 100 g/l sterile skim milk powder. After 2 hours incubation at 37 ± 1°C, add 0.018 g/l brilliant green. Continue incubating for an additional 16 ± 2 hours.
4. Automated workflow: After incubation of the enrichment culture, dispense 500 µl aliquots into 2 ml microtubes and start the automated QIAsymphony DNA extraction protocol.

Manual workflow: After incubation of the enrichment culture, dispense 1 ml aliquots into 2 ml SafeSeal or screw cap tubes and start the manual DNA extraction protocol.

Automated Workflow

Protocol: Automated isolation of bacterial DNA on the QIAsymphony SP

Procedure

1. Close all the drawers and hoods of the QIAsymphony SP/AS instrument.
2. Switch on the instrument and wait until the “Sample Preparation” screen appears and the initialization procedure has finished.
3. Log in to the instrument.
4. Ensure the “Waste” drawer is prepared properly, and perform an inventory scan of the “Waste” drawer, including the tip chute and liquid waste. Replace the tip disposal bag, if necessary.
5. Load the required elution rack into the “Eluate” drawer and perform an inventory scan of the “Eluate” drawer.
6. Load the required reagent cartridge(s) and consumables into the “Reagents and Consumables” drawer.

7. Press the "R+C" button in the touchscreen to open the screen that shows the consumables status ("Consumables/8-RodCovers/Tubes/ Filter-Tips/Reagent Cartridges"). Press the "Scan Bottle" button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Press the "OK" button.
8. Perform an inventory scan of the "Reagents and Consumables" drawer.
9. Place the samples into the appropriate tube carrier and load them into the "Sample" drawer.
10. Using the touchscreen, enter the required information for each batch of samples to be processed.
11. Choose elution volumes according to Table 5.

Table 5a. Sample volumes for QIAsymphony SP mericon purification (AOAC-RI PTM)

Matrix	Elution volume
Peanut butter	200 µl
Spinach	200 µl
Eggs	200 µl
Non-fat dried milk	200 µl
Whole milk	200 µl
Chicken carcass rinses	400 µl
Chocolate (milk)	400 µl
Ground beef (30% fat)	400 µl

Table 5b. Sample volumes for QIAsymphony SP *mericon* purification (NF VALIDATION)

NF VALIDATION specification	Elution Volume
Automated workflow all samples	200 µl

12. **Press the "Run" button to start the purification procedure.**
13. **When sample processing is complete, perform a direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation). Press "Transfer" to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS.**
14. **If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.**
15. **Discard used sample tubes, plates, and waste according to your local safety regulations and replace the tip disposal bag.**
16. **Close the instrument drawers, and proceed with assay setup on the QIAsymphony AS (page 19).**
17. **Clean the QIAsymphony SP during the assay setup on the QIAsymphony AS, or later.**

Note: For daily maintenance, remove the waste bottle, tip park station, tip chute, tip guards, and magnetic-head guards and soak these in a glyoxal and quaternary ammonium salt-based disinfectant (e.g., gigasept® instru AF) for at least 15 minutes. Rinse with water and wipe dry with paper towels. Wipe the QIAsymphony SP worktable and touch screen with an ethanol-based disinfectant (e.g., mikrozid®) then wipe with a damp cloth and dry with a paper towel. For more information, please refer to the QIAsymphony Instrument User Manuals.

Protocol: Assay setup on the QIAsymphony AS

Things to do before starting

- **24 sample kit**: Add 130 µl Multiplex PCR Master Mix (tube[s] with blue lid) to each vial of *mericon* Assay (yellow lid). Transfer the reconstituted *mericon* Assay to a labeled, fresh 2 ml microtube.
- **96 sample kit**: Add 1040 µl Multiplex PCR Master Mix (tube with blue lid) to the vial of *mericon* Assay (yellow lid).
- Dissolve the dried Positive Control DNA (red lid). For both kit sizes add 200 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix. Transfer the reconstituted Positive Control to a labeled, fresh 2 ml microtube.

Procedure

1. **Insert the tip chute into its position on the right hand side in the front part of the QIAsymphony AS module.**
2. **Install an empty tip disposal bag in the bag holder under the "Assays" drawer.**

3. **Switch user interface from sample preparation to assay setup.**
4. **Start the assay definition process.**
5. **For integrated operation (elution rack is automatically transferred from the QIAsymphony SP into the AS module) the "Sample Rack(s)" screen will appear directly.**
6. **All stored sample information (sample status, sample ID, sample volume, and rack ID) is transferred to the QIAsymphony AS module together with the elution rack and will automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface.**
7. **If the assay setup is independent from a former QIAsymphony SP run, select the rack file of the corresponding QIAsymphony SP run or select the rack type of your elution rack for the highlighted "Sample" position (slot 2) and then either manually type in the "Rack ID" of the elution rack or choose "Automatic ID" for a new ID.**
8. **In the "Sample Rack Layout" screen of the assay setup user interface, the elution rack in slot 2 is pictured.**

For integrated operation, or for independent operation in combination with a loaded rack file, sample IDs and sample volumes are automatically assigned to the corresponding positions.

For independent operation without a rack file, select the positions to be processed from the elution rack. Define the highlighted positions as "Sample" then reselect the defined samples and assign sample volumes.

9. **In the "Assay Selection" screen, select the Assay Parameter Set(s) to use in the run.**
10. **In the "Assay Assignment" screen, assign the Assay Parameter Sets to samples.**
11. **In the "Assay Rack(s)" screen, define the assay rack ID. Either type in the assay rack ID manually or choose "Automatic ID" for a new ID.**
12. **The cooling of samples and reagents will start automatically. Check the temperature of the cooling positions.**
13. **The "Loading Information" screen displays the working table of the QIAsymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.**
14. **Place the reconstituted *mericon* Assay(s), the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters.**

15. **Open the “Eluate and Reagents” and “Assays” drawers.**
16. **Load the prepared reagent adapter into slot 3 of the “Eluate and Reagents” drawer according to the illustration in the “Loading Information” screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the “Assays” drawer.**
17. **Load disposable filter-tips into the “Eluate and Reagents” and “Assays” drawers, according to the required number of each tip type.**
18. **Close the “Eluate and Reagents” and “Assays” drawers.**
19. **Upon closing each drawer, press “Yes” to start the inventory scan for each drawer.**
20. **Press “Queue”. Monitoring of the cooling starts.**
21. **Press “Run” to start the run.**
22. **After the run is finished, press “Remove” in the assay setup “Overview” screen. Open the “Assays” drawer and unload the PCR assay adapter.**
23. **Download the result and cycler files via the QIAsymphony Management Console (QMC).**
24. **Proceed to “Protocol: PCR and data analysis on the Rotor-Gene Q“, page 24.**
25. **Perform the regular maintenance/cleaning of the QIAsymphony AS during the PCR run on the Rotor-Gene Q, or later.**

For more information about regular cleaning procedures, please refer to the QIAsymphony Instrument User Manuals.

Manual Workflow

Protocol: Manual isolation of DNA using the *mericon* DNA Bacteria Kit

Things to do before starting

- Prewarm a Thermomixer or heating block to 100°C for use in step 4.

Procedure

1. **Pipet 1 ml enrichment culture into a 2 ml microcentrifuge SafeSeal or screw-cap tube (not supplied) and centrifuge at 13,000 x *g* for 5 minutes.**
2. **Discard the supernatant using a pipet taking care to not disrupt the pellet.**
3. **Add 200 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.**
4. **Place the microcentrifuge tube into a heating block or thermal shaker (800 rpm) set to 100°C. Heat the sample for 10 minutes.**
5. **Remove the sample and allow it to cool to room temperature (15–25°C) for 2 minutes.**
6. **Centrifuge the tube at 13,000 x *g* for 5 minutes.**
7. **Transfer 100 µl of the supernatant to a fresh 1.5 ml microcentrifuge tube. For the PCR reaction, use an aliquot of the collected supernatant diluted according to Table 6.**

Table 6a. Sample volumes for manual mericon assay setup (AOAC-RI PTM)

Matrix	DNA dilution
Peanut butter	Undiluted
Spinach	Undiluted
Eggs	1:50
Non-fat dry milk	Undiluted
Whole milk	Undiluted
Chicken carcass rinses	1:50
Chocolate (milk)	1:50
Ground beef (30% fat)	1:50

Table 6b. Sample volumes for manual *mericon* assay setup (NF-VALIDATION)

Matrix	DNA Dilution
All samples	1:10

Protocol: Manual assay setup

Things to do before starting

- Please refer to "General Precautions for Real-Time PCR Assays", page 8.
- PCR loading block should be stored refrigerated to ensure that PCR setup is performed under stable thermal conditions.
- **24 sample kit**: Add 130 µl Multiplex PCR Master Mix (tube[s] with blue lid) to each vial of *mericon* Assay (yellow lid). Transfer the reconstituted *mericon* Assay to a labeled, fresh 2 ml microtube.

96 sample kit: Add 1040 µl Multiplex PCR Master Mix (tube with blue lid) to the vial of *mericon* Assay (yellow lid).

- Dissolve the dried Positive Control DNA (red lid). For both kit sizes and all cyclers add 200 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix.

Procedure

1. **Place the desired number of PCR 72-well strip tubes into the adapters of the cooling block for the Rotor-Gene Q.**
2. **Set up the sample and control reactions according to Table 7.**
3. **Add reconstituted assay to the tubes first, then add the Sample DNA or controls.**

Table 7. Setup of sample and control reactions

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl
Sample DNA	10 µl	–	–
Dissolved Positive Control DNA	–	10 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	10 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

Real-Time PCR

Protocol: PCR and data analysis on the Rotor-Gene Q

Procedure

1. **Seal the Rotor-Disc after automated PCR setup, or close the strip tubes after manual PCR setup. Place Rotor-Disc or strip tubes in the respective rotor and make sure to apply the locking ring. Place the rotor in the reaction chamber of the Rotor-Gene Q.**
2. **Transfer the cycler file from the QIAsymphony AS to the Rotor-Gene Q.**
3. **Program the thermal cycler according to Table 8.**
4. **Ensure that 'Perform Optimisation Before 1st Acquisition' in the Gain Optimisation menu is activated.**
5. **Start the PCR run.**
6. **Proceed to "Analyzing the Results", page 26.**

Table 8. Cycling protocol for Rotor-Gene Q

Step	Time	Temperature	Comments
Initial PCR activation step	5 mins	95 deg	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling: Denaturation Annealing Extension	 15 secs 15 secs 10 secs	 95 deg 60 deg 72 deg	Data collection at 60°C
Number of cycles	40		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM™	495/520 nm	Cycling A Green
Internal control	MAX™	524/557 nm	Cycling A Yellow

Analyzing the Results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. Figures 1–3 are examples of possible outcomes, which are summarized in Table 9 (page 27).

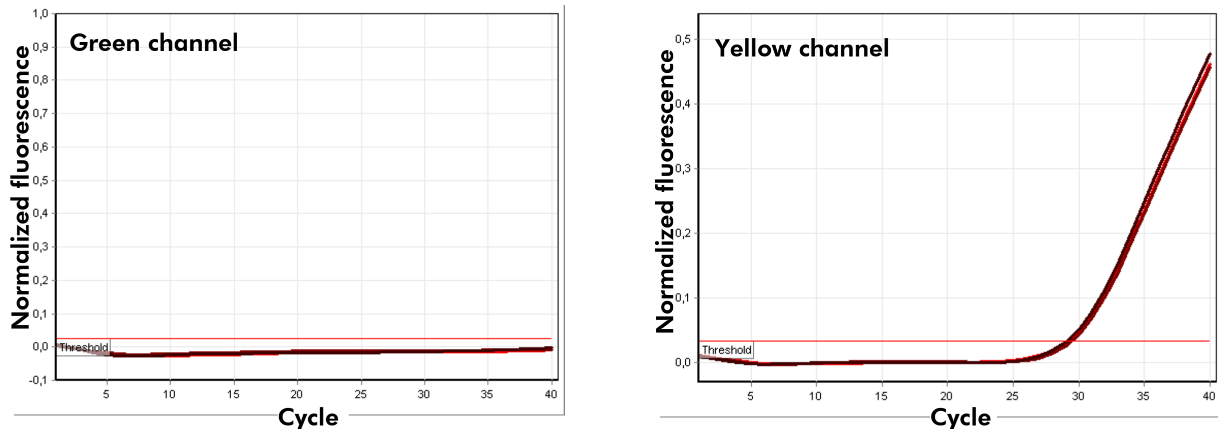

Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

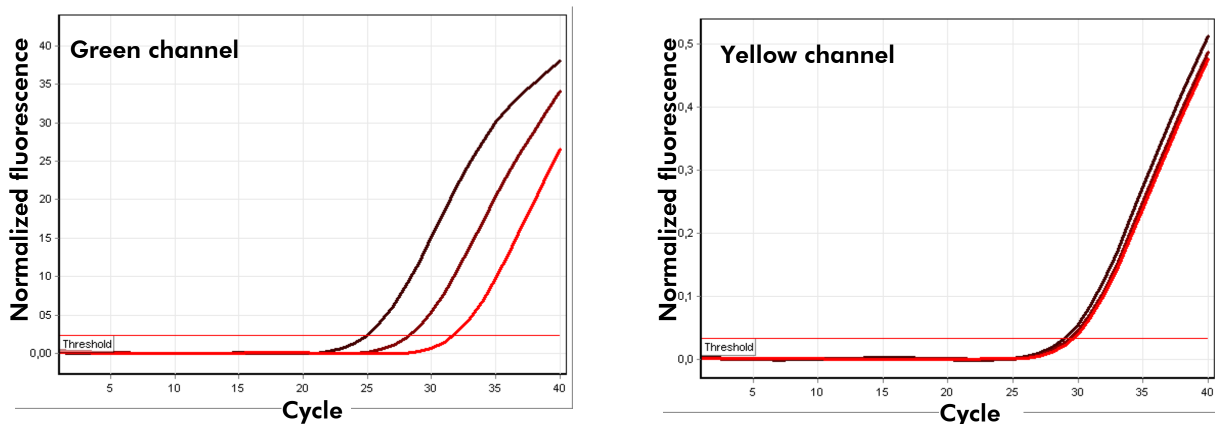

Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

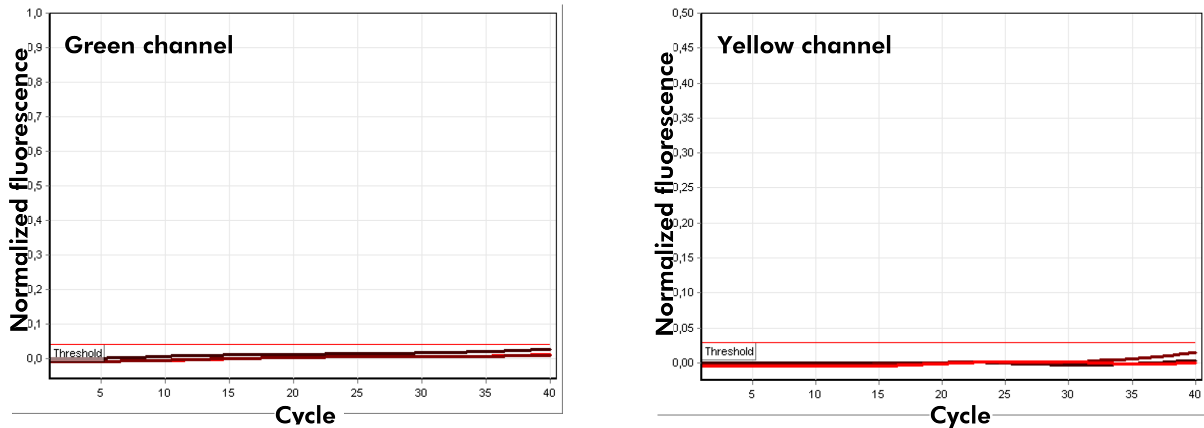

Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 9. Summary of possible outcomes

Amplification of internal control	Amplification of sample	Result
+	+	Sample is positive
+	–	Sample is negative
–	–	PCR failed

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the internal control to higher C_T values. As a guideline, the uninhibited internal control should give a C_T value ranging between 28 and 32. A C_T above 33 indicates inhibition.

In the event of PCR inhibition, further dilute samples 1:10 with RNase-free water and repeat the test.

If DNA template concentration is very high, a shift of the Internal Control to lower cycle values might occur, which does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

Notes

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