February 2015

Product Insert for the AOAC-RI PTM-certified *mericon*® Automated and Manual Listeria monocytogenes and Listeria Species Detection Workflows

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QIAGEN®

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.

Contents

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 7 for "Safety Information".

† A Reuse Seal Set contains 8 Reuse Seal Strips.

DNA extraction — manual workflow

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

Real-time PCR — automated and manual workflows

		(24)	(96)
***mericon* L. monocytogenes Kit**		**290023**	**290025**
***mericon* Listeria spp Kit**		**290123**	**290125**
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 at room temperature (15–25°C). Pathogen Lysis Tubes L (*mericon* DNA Bacteria Plus Kit only) should be stored dry at room temperature (15–25°C). Under these conditions, the kit remains stable for 2 years.

The *mericon* L. monocytogenes and Listeria spp Kits are shipped on dry ice. The Multiplex PCR Master Mix should be stored immediately at –15°C to –30°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 –15°C to –30°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* detection workflows for *L. monocytogenes* and *Listeria* spp. are designed to be used by qualified users in microbiology laboratories for the determination of the presence or absence of *L. monocytogenes* and *Listeria* spp. in food products or on environmental surfaces.

Applicability

The *mericon* detection workflows for *L. monocytogenes* and *Listeria* spp. (*L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. grayi*, and *L. seeligeri*) have been evaluated in an independent laboratory for use with the following food matrices: hot dogs, sliced turkey, smoked salmon, bean sprouts, gouda cheese, mozzarella cheese, and pasteurized whole milk; and the following environmental surfaces: stainless steel, sealed concrete, plastic, and ceramic tile. The protocol includes preparation of an enrichment culture; followed by a manual or automated purification of *L. monocytogenes* and *Listeria* spp. DNA;

and a real-time PCR assay for the presence or absence of pathogen DNA using the *mericon* L. monocytogenes and Listeria spp Kits 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.

Work with samples potentially contaminated with *L. monocytogenes* should be performed in laboratories meeting Biosafety Level 2 (BSL2) regulations. *L. monocytogenes* should not be handled by pregnant women, children, the elderly, or immunocompromised individuals due to the high infection and fatality rates associated with these groups.

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. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

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

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

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). Technical Service in North America can be reached at 1-800-362-7737 and at QIAGEN GmbH at +49-2103-29-12400.

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.

Principle of the assay

The *mericon* L. monocytogenes Assay and mericon Listeria spp Assay are multiplex PCR assays that amplify both a specific DNA target and an internal control with high specificity. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run. Each *mericon* PCR Assay includes a PCR primer set for a pathogen-specific target sequence, probes labeled with two distinct fluorescent dyes (FAM and MAX NHS Ester), positive control DNA, and all of the reagents necessary to perform the analysis. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStarTaq *Plus* DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast-cycling technology including Q-bond. [1]

Data Analysis

The Rotor-Gene Q cycler produces raw data files that are further interpreted by the software using mathematical algorithms to characterize samples. The software guides the user through each step and provides simplicity for beginners as well as an experimental platform for advanced users. [2]

General Precautions for Real-Time PCR Assays

The pathogen detection assays for *L. monocytogenes* and *Listeria* spp. involve DNA detection using PCR. Care must be taken to avoid contamination of the PCR mix.

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 or supplies (e.g., rotors, loading blocks, tubes, and pipets) without gloves.
- In the case of contamination, laboratory benches, apparatus, and pipets can be decontaminated 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 potentially containing pathogens 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 food enrichment cultures

- Oxoid Novel Enrichment (ONE) broth-listeria (CM1066)
- ONE broth-listeria selective supplement (SR0234)
- Lab paddle blender (e.g., Stomacher® 400 Circulator, Seward)*
- Filter homogenizer bags (e.g., VWR®, cat. no. 129-9874)
- Balance

For the preparation of environmental surface enrichment cultures

- Environmental Sponges pre-moistened with Dey-Engley neutralizing broth (e.g., 3M HS10DE2G)
- Environmental Swabs pre-moistened with Dey-Engley neutralizing broth (e.g., 3M RS96010DE)
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)
- Oxoid Novel Enrichment (ONE) broth-Listeria (CM1066)
- ONE broth-Listeria selective supplement (SR0234)

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)

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

Consumables for the QIAsymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Microtubes 2 ml, PP, CB with screw-caps(cat no. 990382)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Elution Microtubes CL with cap strips (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)

For assay setup

- QIAsymphony AS instrument (cat. no. 9001301)*
- *mericon* L. monocytogenes Kit (cat no 290023 or 290025) or Listeria spp Kit (cat. nos. 290123 or 290125)

Accessories and adapters for the QIAsymphony AS

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

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, CB with screw-caps (cat.no. 990382)
- Tip disposal bags (cat. no. 9013395)

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

Manual Workflow

For the preparation of food enrichment cultures

- Oxoid Novel Enrichment (ONE) broth-Listeria (CM1066)
- ONE broth-listeria selective supplement (SR0234)
- 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 Plus Kit (cat. no. 69534)
- 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

For assay setup

- 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)

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

Real-Time PCR

- Rotor-Gene Q 2plex Platform (cat. no. 9001550) or higher*
- Rotor-Gene Q software version 2.3

Specifications of the AOAC-RI PTM-Certified Detection Workflow for *L. monocytogenes* and *Listeria* spp.

The automated and manual workflows for the detection of *L. monocytogenes* and *Listeria* spp. have received AOAC-RI PTM Certification. The specifications for these workflows can be found in Tables 1 and 2. For the PCR assay setup, elution volumes for the automated workflow, and eluate dilutions for the manual workflow, see Table 3.

Confirmation of Positive Results

All positive results for *L. monocytogenes* and/or *Listeria* spp. obtained with the *mericon* detection workflow are presumptive positives and require confirmation by a reference method. Presumptive positive results should be confirmed by the appropriate standard method for the determination of the presence of *L. monocytogenes* and/or *Listeria* spp., starting with secondary selective enrichment. For environmental samples and for testing hot dogs and deli turkey, the confirmation method is USDA/FSIS-MLG 8.09. For bean sprouts and smoked salmon, the confirmation method is FDA/BAM Chapter 10. For milk and dairy products, the confirmation method is AOAC 993.12.

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

Table 1. Overview of the specifications

AOAC-RI PTM-certified specification	Details
Target	*L. monocytogenes* or *Listeria* spp.
DNA extraction kit	Automated workflow: QIAsymphony *mericon* Bacteria Kit Manual workflow: *mericon* DNA Bacteria Plus Kit
Real-time PCR assay	*mericon* L. monocytogenes Kit *mericon* Listeria spp Kit
Enrichment broth	ONE broth with supplement (Oxoid)
Enrichment temperature	30 ±1°C
Enrichment time	24 ± 2 hours
Homogenizer bag	Filter bag
Sample matrices	Hot dogs Sliced turkey Smoked salmon Mung bean sprouts Pasteurized whole milk Gouda cheese Mozzarella cheese
Environmental surfaces	Stainless steel Sealed concrete Plastic Ceramic tile

Table 2. Limit of detection

Workflow segment	Limit of detection*
Overall automated and manual workflow	10^4 cfu/ml
mericon L. monocytogenes Kit *mericon* Listeria spp Kit	10 copies/reaction

* Limit of Detection is derived from method development studies.

Table 3. Sample volumes for *mericon* assay setup

Matrix	Manual workflow eluate dilution	Automated workflow elution volume
Hot dogs	Undiluted	400 µl
Sliced turkey	1:10	400 µl
Bean sprouts	1:10	400 µl
Smoked salmon	Undiluted	200 µl
Pasteurized whole milk	Undiluted	200 µl
Gouda cheese	1:50*	400 µl
Mozzarella cheese	1:50*	400 µl
Stainless steel	Undiluted	200 µl
Sealed concrete	Undiluted	200 µl
Ceramic tile	Undiluted	200 µl
Plastic	Undiluted	200 µl

* Dilute to 1:100 if IC inhibited.

Protocol: Preparation of Enrichment Cultures from Food Matrices

Procedure

1. **Add 25 g of the potentially contaminated food sample to a filter homogenizer bag.**
2. **Add 225 ml Oxoid ONE Broth Listeria plus ONE broth-listeria selective supplement to the bag.**
3. **Homogenize the food sample using a lab paddle blender at 230 rpm for 1.5 min (± 10 s). Then, seal the homogenizer bag and incubate the homogenate at 30 ± 1°C for 24 ± 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.

Protocol: Preparation of Enrichment Cultures from Environmental Surfaces

Procedure

1. **Using Dey-Engley neutralizing broth (DE), pre-moisten environmental sponges (10 ml DE) or swabs (1 ml DE).**
2. **Sample surface areas using vertical and horizontal motions.**
3. **Leave the sponge or swabs at room temperature (24 ± 2°C) for 2 hours prior to analysis.**
4. **Place the sponge samples into a homogenizer bag containing 225 ml of Oxoid ONE Broth Listeria plus ONE broth-Listeria selective supplement and incubate at 30 ± 1°C for 24 ± 2 hours.**
5. **Add the swabs to tubes containing 10 ml of Oxoid ONE Broth Listeria plus ONE broth-Listeria selective supplement and incubate at 30 ± 1°C for 24 ± 2 hours.**
6. **Automated workflow: After incubation of the enrichment culture, dispense 500 µl aliquots into 2 ml microtubes and start the automated QIAsymphony DNA extraction protocol.**
7. **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 4.

Table 4. Sample volumes for *mericon* assay setup

Matrix	Elution volume
Hot dogs	400 µl
Sliced turkey	400 µl
Bean sprouts	400 µl
Smoked salmon	200 µl
Pasteurized whole milk	200 µl
Gouda cheese	400 µl
Mozzarella cheese	400 µl
Stainless steel	200 µl
Sealed concrete	200 µl
Ceramic tile	200 µl
Plastic	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 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 (see below).**
17. **Clean QIAsymphony SP during or after assay setup on QIAsymphony AS.**

    Note: For daily maintenance, remove the waste bottle, tip park station, tip chute, tip guards, and magnetic-head guards and soak in a glyoxal and quaternary ammonium salt-based disinfectant (e.g., gigasept® instru AF) for at least 15 min. 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. **On 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. **On the "Assay Selection" screen, select the Assay Parameter Set(s) to use in the run.**
10. **On the "Assay Assignment" screen, assign the Assay Parameter Sets to samples.**
11. **On 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 26.**
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 Plus Kit

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 min (± 10 s).
2. Discard the supernatant using a pipet, taking care to not disrupt the pellet.
3. Add 400 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.
4. Transfer the entire mixture to a Pathogen Lysis Tube (supplied). Tightly cap the tube, secure it vertically or horizontally to a vortex adapter, and vortex at maximum speed for 10 min.
5. Centrifuge the tube at 13,000 x *g* for 5 min (± 10 s).
6. 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 5.

Table 5. Sample volumes for *mericon* manual assay setup

Matrix	DNA dilution
Hot dogs	Undiluted
Sliced turkey	1:10
Bean sprouts	1:10
Smoked salmon	Undiluted
Pasteurized whole milk	Undiluted
Gouda cheese	1:50*
Mozzarella cheese	1:50*
Stainless steel	Undiluted
Sealed concrete	Undiluted
Plastic	Undiluted
Ceramic tile	Undiluted

* Dilute to 1:100 if IC inhibited.

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).
- **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 6.**
3. **Add reconstituted assay to the tubes first, then add the Sample DNA or controls.**

Table 6. 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. If using strip tubes, fill any unused positions with empty tubes. 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 7.**
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 27.**

Table 7. Cycling protocol for the Rotor-Gene Q

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	15 s	60°C	
Extension	10 s	72°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 8 (page 28).

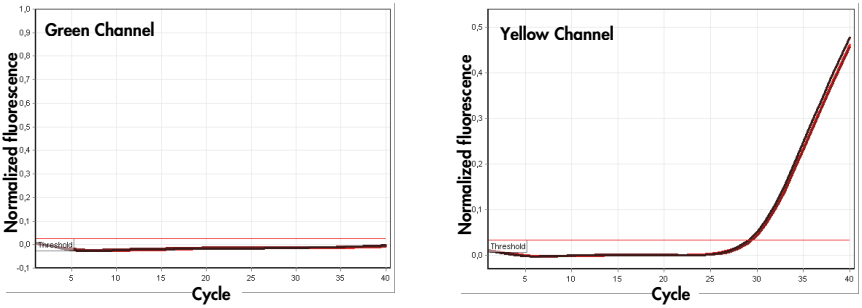

Figure 1. The sample is negative for the tested pathogen. The 3 sample curves in the Green Channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the Yellow Channel (right) are above the threshold, indicating that the PCR was successful.

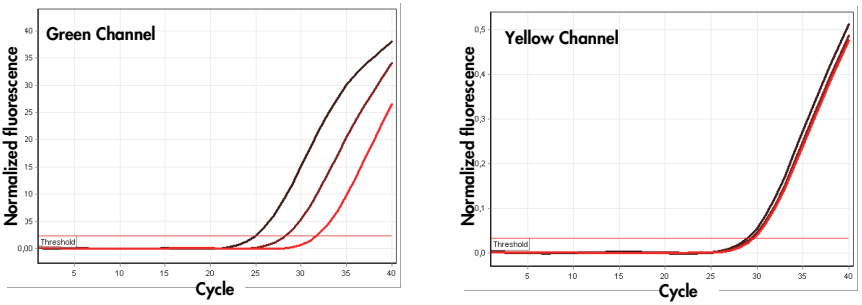

Figure 2. The sample is positive for the tested pathogen. The 3 sample curves in the Green Channel (left) and the corresponding curves of the internal control in the Yellow Channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

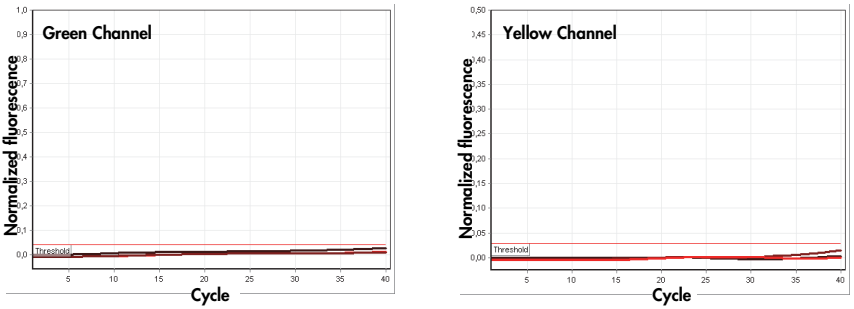

Figure 3. The PCR is inhibited. There is no amplification of the three samples in the Green Channel (left) or the internal control in the Yellow Channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 8. Summary of possible outcomes

Amplification of Internal control	Amplification of sample	Result
C_T 28-32	+	Sample is positive
C_T 28-32	–	Sample is negative
C_T 32.01–40.00	+ / –	PCR inhibited

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 32.01 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.

Software version 2.3 has a graphical user interface to version 2.2. It provides easily interpretable information regarding the presence or absence of pathogen DNA. Figure 4 shows how the indicated results symbols in Table 9 (page 29) are presented onscreen.

Table 9. Result symbols from software version 2.3

Symbol	Result
+	Target detected
–	Target not detected
?	Undetermined
X	Invalid
!	Warning

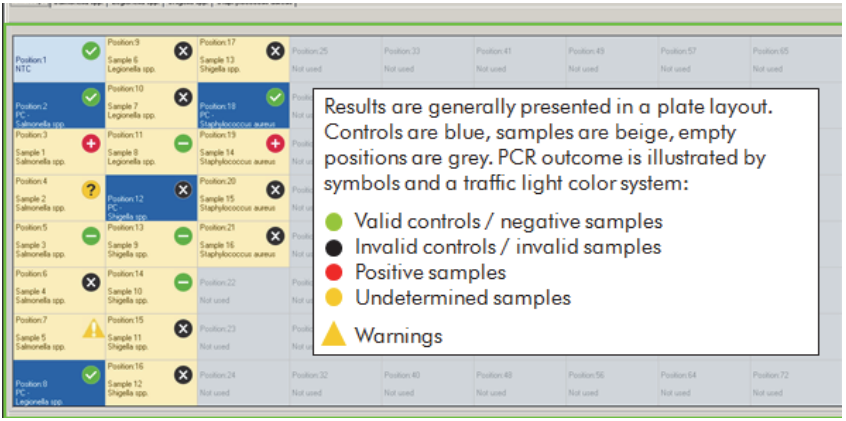

Figure 4. An example of a Results Table (in software version 2.3). The symbols are added to the results table, indicating the absence of the pathogen (green symbol), the presence of the pathogen (red symbol), undetermined samples (yellow symbol), or invalid samples (black symbols). The black and yellow symbols indicate that user attention is required.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center http://b2b.qiagen.com/knowledge-and-support/troubleshooting-and-support/. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Issue	Comments and suggestions
No signal with positive control	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol.	For data analysis, select the Green Channel (FAM) for the samples and the Yellow Channel (VIC or corresponding filter set) for the internal control. See Table 7 on page 26.
b) Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. Table 7 on page 26.
c) Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 6 on page 25. Repeat the PCR, if necessary.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage and Shelf Life" (page 5).	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *mericon* PCR Assay has expired.	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal in the internal amplification control	
a) The PCR conditions do not comply with the protocol.	Check that PCR conditions match the cycling protocols in Table 6 on page 25. Repeat the PCR with corrected settings, if necessary.

Issue	Comments and suggestions
b) The PCR was inhibited.	Use the recommended DNA isolation method in this workflow. If there is inhibition, dilute the DNA sample and repeat the PCR.
c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage and Shelf Life" (page 5).	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
d) The *mericon* PCR Assay has expired.	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals present for the negative controls	
a) Contamination occurred during PCR setup.	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after the addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that the work space and instruments are decontaminated at regular intervals.
b) Contamination occurred during extraction.	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that the work space and instruments are decontaminated at regular intervals.

Notes

Notes

Notes

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