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

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NF
VALIDATION
EN ISO 16140

QIA 36/01 – 02/13
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

Contents

Kit Contents

DNA extraction — automated workflow

QIAsymphony® *mericon* Bacteria Kit	**(360)**
Catalog no.	**931156**
No. of reactions	**360**
Reagent Cartridge*	2
Piercing Lid	2
TopElute Fluid	60 ml
Reuse Seal Set†	2

* Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† A Reuse Seal Set contains 8 Reuse Seal Strips.

DNA extraction — manual workflow

Catalog no.	***mericon* DNA Bacteria Kit (100)**	***mericon* DNA Bacteria Plus Kit (50)**
No. of reactions	**69525** **100**	**69534** **50**
Fast Lysis Buffer	1 x 25 ml	2 x 25 ml
Pathogen Lysis Tubes L	–	5 x 10

Real-time PCR — automated and manual workflows

***mericon* Salmonella spp Kit**		**(96)**
Catalog no.		**290015**
No. of reactions		**96**
Yellow	*mericon* Assay*	1 x 96 reactions
Red	Positive Control DNA	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml
	RNase-Free Water	1.9 ml
Blue	Multiplex PCR Master Mix†	1040 µl
	50x ROX Dye Solution	210 µl

* 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).

Shipping and Storage

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 –30 to –15°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 –30 to –15°C for long-term storage.

Intended Use

Products for the *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.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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 meat products, seafood, and vegetables, egg products, animal feed, and environmental samples (excluding primary production stage environment).

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 meat products, seafood, and vegetables, egg products, animal feed, 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.

For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate 36/01-02/13 available at **http://nf-validation.afnor.org/en** or upon request from QIAGEN.

Users should comply with Good Laboratory Practices (EN ISO 7218 standard).

In the context of NF Validation, test portions weighing more than 25 g have not been tested.

In the context of NF Validation, the QIAGEN Rotor-Gene Q 5plex HRM System (e.g., cat. no. 9001650) and Software version Rotor-Gene Q 2.3.1.49 have been validated. There is a newer version of the software (Rotor-Gene Q 2.3.5) applicable to the product, but this version hasn't been validated.

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. The initial ambient temperature of the laboratory 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 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 	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.

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.

Principle of the Assay

The *mericon* Salmonella spp. Assay is a multiplex PCR assay that amplifies 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 *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 pipettes for the PCR master mix and the DNA samples. Use of pipette 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, pipettes) without wearing gloves.
- In case of contamination, laboratory benches, apparatus, and pipettes can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution. Afterwards, the benches and pipettes 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAsymphony® *mericon* Bacteria Kit, *mericon* DNA Bacteria Kit, and *mericon* Salmonella spp. Kit is tested against predetermined specifications to ensure consistent product quality.

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, samplecarr. (24), Qsym (cat. no. 9242083)
- Cooling Adapter, EMT, v2, Qsym (cat. no. 9020730)

Consumables for the QIAsymphony SP

- Sample Prep Cartridges, 8-well (336) (cat. no. 997002)
- 8-Rod Covers (144) (cat. no. 997004)
- Microtubes 2 ml, PP, without lids (Sarstedt®, cat. no. 72.608)
- Filter-Tips, 1500 µl (1024) (cat. no. 997024)
- Elution Microtubes CL (24 x 96) (cat. no. 19588)
- Tip disposal bags (15) (cat. no. 9013395)

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

For assay setup

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

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 (1024) (cat. no. 990332)
- Filter-Tips, 50 µl (1024) (cat. no. 997120)
- Micro tubes 2 ml, PP, without lids (Sarstedt, cat. no. 72.608)
- Tip disposal bags (15) (cat. no. 9013395)

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

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 (100) (cat. no. 69525) or *mericon* DNA Bacterial Plus Kit (50) (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.
- Pipettes and pipette tips

For assay setup

- Pipettes and filter pipette tips

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

- Loading Block 72 x 0.1 ml Tubes (cat. no. 9018901) or Loading Block 72 x 0.1 ml Multi-channel (cat. no. 9018902)
- Strip Tubes and Caps, 0.1 ml (250) (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.

For Real-Time PCR

- Rotor-Gene Q 2plex Platform (cat. no. 9001550)*
- Rotor-Gene Q 5plex HRM Platform (cat. no. 9001580) as alternative to the 2plex platform
- Rotor-Gene Q software version 2.3.5 (version 2.3.1.49 is the last version validated)
- Assay package 3.0.4 for *mericon*-specific mode

* 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 Table 1 and Table 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 ± 1°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 NF Validation *Salmonella* spp. Detection Workflow

QIAGEN *mericon* Salmonella spp. automated and manual workflows are validated in the context of NF VALIDATION, with the EN ISO method taken as a reference (EN ISO 6579) and validation protocol implemented (EN ISO 16140-2, 2016) for the detection of *Salmonella* spp. in meat products, seafood, and vegetable products, egg products, animal feed, and environmental samples (excluding primary production stage environment). For more information about the end of validity of the NF VALIDATION, please refer to the certificate QIA 36/01 – 02/13 available at **http://nf-validation.afnor.org/en** or upon request to QIAGEN.

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 6579-1 and ISO 6579-1/A1 and of EN ISO 6887 standards.

The specifications for these workflows can be found in Table 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. Partial PCR inhibitions were observed for 2.1% (manual extraction) and 0.7% (automated extraction of the analyzed samples. Table 4c clearly shows the way to solve these outcomes.

In the context of NF Validation, the QIAGEN Rotor-Gene Q 5plex HRM (e.g., cat. no. 9001650) and Software version Rotor-Gene Q 2.3.1.49 have been validated. There is a newer version of the software (Rotor-Gene Q 2.3.5) applicable to the product, but this version hasn't been validated.

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	Meat Products Seafood Vegetable Products Egg Products Animal feed 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 of inhibited samples for PCR Assay

NF VALIDATION specification	Details
Manual workflow	1:10 or 1:50 dilution of inhibited DNA eluate
Automated workflow	**QIAsymphony** 200 µl elution volume

Confirmation of Positive Results

In the context of NF VALIDATION, all samples identified as positive by the *mericon* pathogen detection assay must be confirmed by the standard 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 by this scheme by transferring the original BPW enrichment into RVS & MKTT.

Table 5. Confirmatory workflow of presumptive positive samples

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 Incubation for (24±3) h at (41.5±1) °C	1 ml + 10 ml MKTTn 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 and that shares the same enrichment procedure as Qiagen *mericon Salmonella* can be utilized as a confirmatory method.

In the event of discordant results (presumed positive with QIAGEN *mericon Salmonella*, not confirmed by the tests described above), the laboratory must employ adequate means to ensure the validity of the result obtained, e.g., streaking from RVS and MKTTn media onto XLD, Hektoen, and C8-esterase–based selective agar plates or subculturing into MSRV.

In the scope of NF validation, the manual method for DNA extraction was found to have a False Positive Ratio (FPR%) of 7.1%.

In the scope of NT validation, inhibitions were observed for 6.8% (33 samples) of the DNA extracts. They were mainly belonging to the vegetable category and specifically aromatic herbs and spices (23 samples) and egg products (5 samples). Dilutions (1:10 or 1:50) of the DNA extracts allowed removing the inhibitions.

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 ± 10 s. Then, seal the homogenizer bag and incubate the homogenate for 18 ± 2 h at 37°C.
3. In the scope of the AOAC-RI PTM validation only (milk chocolate is out of scope of NF validation). 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 h incubation at 37 ± 1°C, add 0.018 g/l brilliant green. Continue incubating for an additional 16 ± 2 h.
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 6.

Table 6a. 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 6b. 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 26).

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 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. 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 using the *mericon-specific* assay package 3.0.4", page 32.
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. Pipette 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
2. Discard the supernatant using a pipette 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 min.
5. Remove the sample and allow it to cool to room temperature (15–25°C) for 2 min.
6. Centrifuge the tube at 13,000 x *g* for 5 min.
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 7a or 7b.

Table 7a. 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 7b. Sample dilutions of inhibited samples for manual *mericon* assay setup (NF-VALIDATION)

Matrix	DNA Dilution
All samples showing inhibition	1:10 or 1:50

Protocol: Manual assay setup

Things to do before starting

- Please refer to “General Precautions for Real-Time PCR Assays”, page 11.
- 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 8.
3. Add reconstituted assay to the tubes first, then add the Sample DNA or controls.

Table 8. 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 using the *mericon*-specific assay package 3.0.4

Procedure

1. Close the strip tubes after manual PCR setup. Place the 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. Open the RGQ *mericon* Data Tool by double clicking on the desktop icon.
3. Open the RGQ software in the *mericon*-specific mode by double clicking on the appropriate *mericon* template. The template applies all cycler settings for the analysis. See Table 9 for cycling parameters used.
4. Ensure and confirm that the locking ring is attached.
5. Start the PCR run by pressing "Start Run".

   Analyze the results with the provided easily interpretable information regarding the presence or absence of pathogen DNA. Figure 2 shows how the indicated results symbols in Table 11 (page 35) are presented on the screen.

Table 9. Cycling protocol for 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

Table 10. Result symbols from *mericon*-specific mode software version 2.3

Symbol	Result	Next step
+	Target detected	Accept - positive
–	Target not detected	Accept - negative
?	Undetermined	repeat analysis
X	Invalid controls or samples (out of range)	Dilute and repeat if IC>32 and target is not detected
!	Warning	Attention required

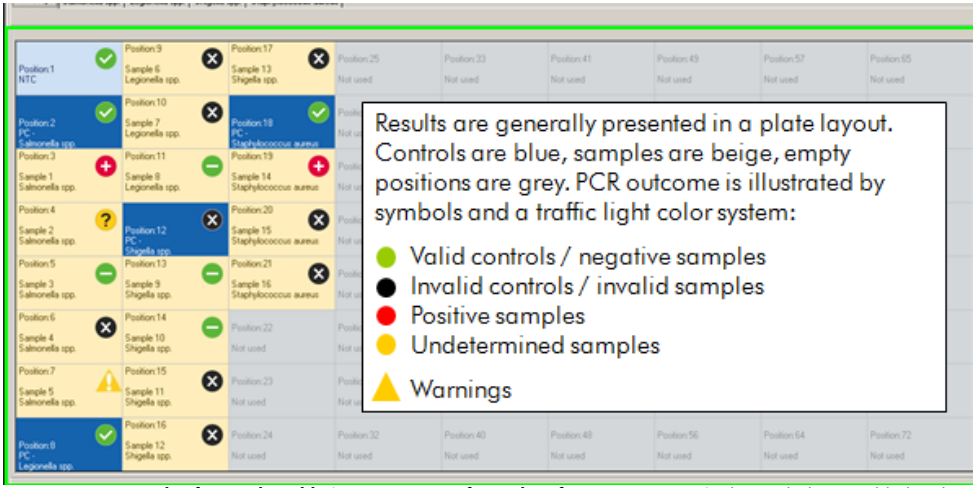

Figure 1. An example of a Results Table (in *mericon*-specific mode software version 2.3). The symbols are added to the results table, indicating the absence of the pathogen (green symbol), the presence of the pathogen (red symbol), undetermined samples (yellow symbol), or invalid samples (black symbols). The black and yellow symbols indicate that user attention is required. Note that invalid controls can be because of inhibition of the PCR and should be diluted and repeated. Alternatively, 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.

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 Figure *2*–Figure *4* are examples of possible outcomes, which are summarized in Table 11 (page 35).

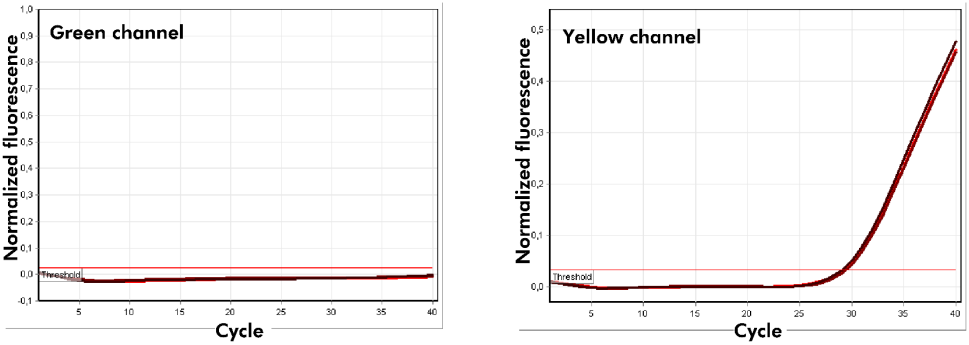

Figure 2. The sample is negative for tested pathogen. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

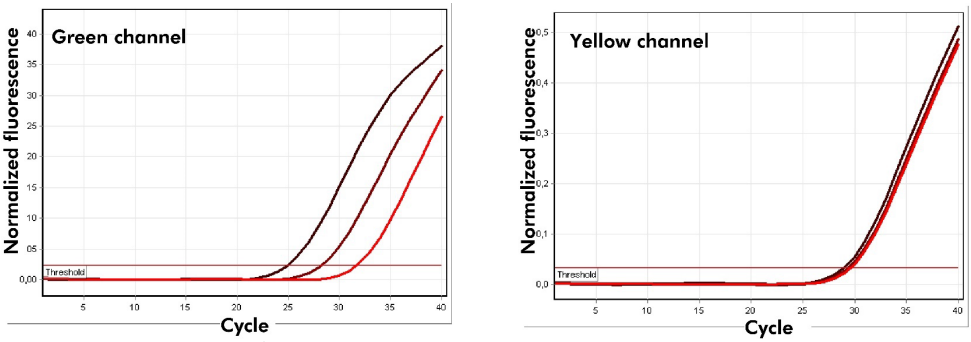

Figure 3. The sample is positive for tested pathogen. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of pathogen DNA in the sample and a successful PCR.

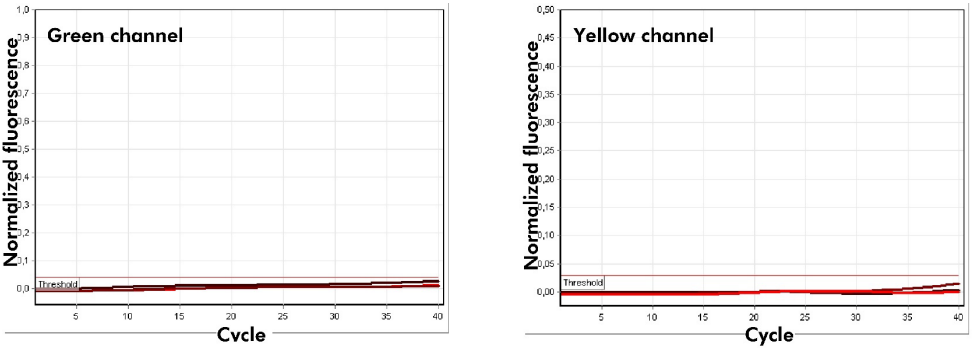

Figure 4. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 11. Summary of possible outcomes

Amplification of sample	Amplification of Internal Control	Result
CT <38	CT 28–32	Positive
CT <38	CT 32, CT < 28, or No CT	Positive
CT range 38.01–40	CT range 28–32	Indeterminate; repeat test
No CT	CT range 28–32	Negative
No CT	CT > 32 or No CT	IC invalid, PCR inhibited; dilute sample and repeat test

If results obtained are indeterminate or inhibited, the sample should be reanalyzed. Samples that produce an invalid IC (CT >32 or No CT) and No CT for the Salmonella target channel can be reanalyzed by diluting the extracted sample 1:10, 1:50 until 1:100 in RNase-free water.

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 CT values. As a guideline, the uninhibited internal control should give a CT value ranging between 28 and 32. A CT above 32 indicates potential inhibition.

In the event of a PCR-inhibited Internal Control (CT >32 or No CT) and a positive sample result, repeating the test is not necessary.

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.

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 in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		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 9 on page 32.
b)	Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See Table 9 on page 32.
c)	Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 8 on page 31. Repeat the PCR, if necessary.
d)	The storage conditions for one or more kit components did not comply with the instructions given in "Shipping and Storage" (page 6)	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 9 on page 32. Repeat the PCR with corrected settings, if necessary.
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.

		Comments and suggestions
c)	The storage conditions for one or more kit components did not comply with the instructions given in "Shipping and Storage" (page 4)	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 pipette the positive controls last. Make sure that the workspace 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 workspace and instruments are decontaminated at regular intervals.

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, 50x ROX Dye Solution	290015
QIAsymphony *mericon* Bacteria Kit (360)	For 360 preparations: 2 Reagent Cartridges, Piercing Lid, TopElute Fluid (60 ml), Reuse Seal Set	931156
Related products		
QIAGEN Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation, and training	9001650
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001580

Product	Contents	Cat. no.
Rotor-Gene Q 2plex Platform	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation, and training not included	9001550
QIAsymphony SP instrument	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAsymphony AS instrument	QIAsymphony assay setup module: includes 1-year warranty on parts and labor	9001301
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Insert, 2.0ml v2, samplecarr. (24), Qsym	Secondary tube adapter (for 2 ml screw-cap tubes, tube insert 3b) for use with the QIAsymphony SP tube carrier	9242083
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020730
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS instruments	997024

Product	Contents	Cat. no.
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Tip disposal bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
Cooling Adapter, Reagent Holder 1, Qsym	Adapter for holding 18 x 2 ml conical tubes, and 6 x 5 ml conical tubes; for use with the QIAsymphony AS (software version 3.1 or higher)	9018090
Adapter 2 x Rotor-Disc, Qsym	Adapter for holding up to 2 x Rotor-Disc 72 on Rotor-Disc 72 Loading Blocks; for use with the QIAsymphony AS (software version 3.1 or higher)	9242204
Rotor-Disc 72 Loading Block	Aluminium block for manual and automated reaction setup in Rotor-Disc 72 discs	9018910
Rotor-Disc 72 (240)	10 x 24 individually wrapped discs for 17,280 reactions of 20–25 µl	981303
Rotor-Disc 72 (24)	24 individually wrapped discs for 1728 reactions of 20–25 µl	981301
Rotor-Disc Heat Sealing Film (600)	10 x 60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981604
Rotor-Disc Heat Sealing Film (60)	60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981601
Rotor-Disc Heat Sealer 110V	Heat sealing instrument for use with Rotor-Discs; requires Rotor-Disc 72 or 100 Loading Block	9018898

Product	Contents	Cat. no.
Rotor-Disc Heat Sealer 230V	Heat sealing instrument for use with Rotor-Discs; requires Rotor-Disc 72 or 100 Loading Block	9019725
Rotor-Disc 72 Locking Ring	For locking a Rotor-Disc 72 in the Rotor-Disc 72 Rotor	9018900
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332
Filter-Tips, 50 µl	Sterile, Disposable Filter-Tips, racked; (8 x 128); for use with the QIAsymphony AS	997120
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
Rotor-Gene Q 72-Well Rotor	For holding Strip Tubes and Caps, 0.1 ml, with reaction volumes of 10–50 µl; requires Locking Ring 72-Well Rotor	9018903
Loading Block, RG Strip Tubes 72, Qsym	Adapter for holding 18 strips of 4 tubes; for use with the QIAsymphony AS (software version 3.1 or higher)	9018092
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions of 10–50 µl	981103
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 ml in the 72-Well Rotor	9018904

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References

1. QIAGEN GmbH. (March 2012). *mericon* Automated Pathogen Detection Workflow Handbook.
2. QIAGEN GmbH. (May 2018). QIAsymphony SP/AS Consolidated Operating Guide.

Document Revision History

Date	Changes
08/2021	Updated the handbook for renewal of AFNOR validation. Removed Product Insert and Quick-Start Protocol in the Kit Contents tables of the *mericon* DNA Bacteria Kit, *mericon* DNA Bacteria Plus Kit, and QIAsymphony® *mericon* Bacteria Kit. Removed references to the *mericon* Salmonella spp. Kit (360), as it has been discontinued. Revised the "Intended User" section (specified food products). Added a new section: Principle of the assay. Added details on NF Validation in the "Applicability" section. Updated the "Specifications of the NF Validation Salmonella spp. Detection Workflow" section. Revised the "Confirmation of Positive Results" section. Revised "Protocol: PCR and data analysis on the Rotor Gene Q" (added reference to specific assay package and software versions). Added a table for result symbols from mericon-specific mode software version 2.3 (Table 1). Added a figure for an example of a Results Table in mericon specific mode software version 2.3 (Figure 1). Revised the table of possible outcomes (Table 10). Inserted the "Troubleshooting Guide" section. Added references to the "mericon Automated Pathogen Detection Workflow Handbook" and the "QIAsymphony SP/AS Consolidated Operating Guide".
04/2022	Removed “dairy products” from the list of validated samples. Added statement/data regarding inhibitions observed from the samples. In Table 10, changed the Internal Control value in the condition to dilute and repeat for invalid controls or samples. In Table 11, changed the CT values for amplification of Internal Control.

Notes

Notes

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