thermo scientific

SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay

USER GUIDE

Lysis and real-time PCR detection of *Campylobacter jejuni, C. coli*, and *C. lari* in food and environmental samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or later

Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software v2.0 or later

Catalog Number A56835

Publication Number MAN0018692

Revision E.0

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 012101

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

Revision history: MAN0018692 E.0 (English)

Revision	Date	Description
E.0	17 January 2024	A new chapter was added Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument (page 27).
D.0	10 July 2023	• A note was added for instrument dye calibration. • The RapidFinder™ Analysis Software version was updated. • The RapidFinder™ Analysis Software and RapidFinder™ Express Software assay file names were updated.
C.0	2 December 2022	• New SKU was created for the kit. • Kit contents were modified—the blue dye moved from the Lysis Reagent 1 (LR1) to Proteinase K (ProK). • Pierceable lysis seals were added to kit contents. • New handling tools—cutting tool, capping tool, and uncapping tool—were added to the Lysis Materials table. • Improved plate/tube products were added to the PCR Materials table. • A new chapter was added, Confirm presumptive colonies using the PCR assay. • The RapidFinder™ Analysis Software version was updated. • Spinning guidelines were added. • Product SKUs were updated. • User guide template was updated with associated updates to the general document organization, limited license information, trademark statement, safety statements, and support information.
B.0	5 February 2021	The instrument software version was updated.
A.0	29 January 2020	New document for SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

Name and intended use

The Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay enables real-time PCR detection of *Campylobacter jejuni, C. coli*, and *C. lari* from food and environmental samples. This kit is for use in laboratories undertaking microbiological analysis. See Table 1 for compatible instruments and software.

Note: The qPCR instrument must be calibrated with the following dyes before use: FAM™, VIC™, ABY™, and JUN™.

Table 1 Instruments and software

PCR instrument[1]	Software	Pathogen Assay File
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument	Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or later	**Campylobacter-Mpx-ST-A56835-QS5-1.1** or later[2]
Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR Instrument	Applied Biosystems™ RapidFinder™ Express Software v2.0 or later	**Campybolacter_Multiplex_SureTect_7500_1.0** or later[2]

[1] or equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries.

[2] Assay files and instructions are available at **thermofisher.com/molecular-microbiology-software**.

Principle of the test

This assay is based on TaqMan™ PCR technology. Dye-labeled probes target unique DNA sequences specific to *Campylobacter jejuni, C. coli*, and *C. lari*, and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. For more information about real-time PCR, go to **thermofisher.com/qpcreducation**.

The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Procedure overview

Enriched food or environmental samples are combined directly with ready-to-use Lysis Reagent 1 and Proteinase K, to lyse bacterial cells present in the sample and release their DNA into solution.

Lysates are transferred to the Campylobacter jejuni, C. coli and C. lari PCR Tubes to rehydrate the lyophilized PCR pellets. The pellets contain lyophilized target-specific primers, dye-labelled probes, and PCR master mix components. The PCR tubes are sealed, loaded into the real-time PCR instrument, then the run is started using the RapidFinder™ software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required.

Results are achieved approximately 80 minutes after loading the prepared sample into the instrument.

Limitations

- The test is designed to detect DNA from target organisms that have been present at a minimum level of 1 CFU/sample, and have grown to detectable levels during the enrichment.
- The customer is responsible for validation of sample matrices or culture media not described in this document.
- When testing a sample type or culture medium that has not been validated, we recommend testing a selection of known negative and positive samples, to ensure that expected results are achieved. See “Test control organisms” on page 38 and EN ISO 22174:2005.
- See Appendix A, “Troubleshooting” for additional information.

Contents and storage

Store the kit protected from light, at 2–8°C. Bring to room temperature (23±5°C) before opening.

Table 2 SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay, 96 tests (Cat. No. A56835)

Contents	Amount	Storage[1,2]
Lysis Reagent 1 Tubes (clear liquid containing fine white particles)	12 strips of 8 tubes	2–8°C
Thermo Scientific™ 96-Well Pierceable Seals	2 sheets	
Proteinase K (blue liquid)	1 tube	
Campylobacter jejuni, C. coli and C. lari PCR Tubes	12 strips of 8 tubes 1 pellet each	
PCR Caps	12 strips of 8 caps	

[1] Store the kit protected from light.
[2] Opening the kit does not affect shelf life.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or **thermofisher.com**. They may also be available through Fisher Scientific (**fisherscientific.com**), MLS, or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Note: Parts may ship separately depending on configuration and storage conditions.

Materials for enrichment

Table 3 Equipment, accessories, and consumables

Item	Source
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
• Homogenizer Laboratory Blender • Diluflux™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm • Diluflux™ Pro Automated Gravimetric Dilutor with robotic dispensing arm	DB5000A DB4100A DB4150A
Sample enrichment bags, one of the following or equivalent:	
• BagFilter™ 400 (400 mL) • BagPage™ 400 (400 mL) • BagLight™ 400 (400 mL) • RollBag™ 1300 (1300 mL)	DB4011A DB4012A DB4013A DB4014A
Incubator fitted with racks for homogenizer bags	**thermofisher.com**
Disposable gloves	MLS
Variable volume single-channel pipette, 1- to 10-mL	Available through the Thermo Fisher Microbiology ordering process.
96-well rack	
Filtered pipette tips, 1- to 10-mL	
Sample tubes, 1.5-mL	

Table 4 Media

Item	Source
Bolton Broth, 500 g of base	CM0983B or equivalent
Oxoid™ Modified Bolton Broth Selective Supplement	SR0208E or equivalent
Buffered Peptone Water (BPW)	MLS

Materials for manual lysis

Table 5 Materials for manual lysis of enriched cultures

Item	Source
Plastics, consumables, and reagents	
Single-channel pipette, 10- to 100-µL *or* Electronic adjustable spacing, multichannel pipette, 10- to 100-µL	Available through the Thermo Fisher Microbiology ordering process.
Single-channel stepper pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	
Domed Lysis Tube Caps	A56895
Thermo Scientific™ 96-Well Pierceable Seals	A55331
MicroAmp™ Adhesive Film Applicator (for pierceable seals)	4333183
Thermo Scientific™ PCR Strip Cutting Tool	A55329
Thermo Scientific™ PCR Capping Tool	A55327
Thermo Scientific™ PCR Uncapping Tool	A55328
Additional materials for the thermal cycler method	
Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
SimpliAmp™ Strip Plate Tray	A55333
Thermo Scientific™ 96-Well PCR Base	A55330

Materials for manual qPCR sample preparation and PCR

Table 6 Materials for manual qPCR sample preparation and PCR

Item	Source
Real-time PCR instrument and accessories, one of the following instrument packages	
QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block, with RapidFinder™ Analysis Software v3.0 or later For use with SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay and Pathogen Assay File: **Campylobacter-Mpx-ST-A56835-QS5-1.1** or later	A36320 (desktop) or equivalent A36328 (laptop) or equivalent Contact your local microbiology sales representative

Table 6 Materials for manual qPCR sample preparation and PCR *(continued)*

Item	Source
7500 Fast Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later For use with SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay and Pathogen Assay File: **Campybolacter_Multiplex_SureTect_7500_1.0** or later	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative
For the QuantStudio™ 5 Food Safety Real-Time PCR Instrument	
QuantStudio™ 5 Strip Plate Tray	A55332
Thermo Scientific™ 96-Well PCR Base	A55330
For the 7500 Fast Food Safety Real-Time PCR Instrument	
Thermo Scientific™ 96-Well PCR Base	A55330
7500 Fast 96W Strip Plate Adaptor	A46347
Armadillo™ Low-Profile PCR Strip Plate, 96-well, clear, pack of 5[1]	AB2696SMP
Ultra Clear qPCR Caps, strips of 8	PT0615
Additional materials for PCR	
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See **thermofisher.com/plastics** for more information.
Plate centrifuge	
8-channel pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	
SimpliAmp™ Strip Plate Tray Cover (optional)	A55971
Thermo Scientific™ PCR Strip Cutting Tool	A55329
Thermo Scientific™ PCR Capping Tool	A55327

[1] Used as balancing tubes and managing the lid pressure if less than 2 full strips are processed.

Materials for automated lysis and qPCR sample preparation

Note: The appliances in Table 7 are used with the RapidFinder™ and SureTect™ Automation Workflow to operate properly.

Table 7 Materials for automated lysis and qPCR sample preparation

Instrument and Appliances	Source
Automation Platform, one of the following:	
Automation Platform CHOICE Head[1] Automation Platform Head R 96[2]	A59017 A66295
SureTect™ Automation Software	A66373 Downloadable from **thermofisher.com**
Instrument item requirements, not included	
Laptop[3]	A49006 or equivalent performing
Automation Starter Pack • Automation Lysis Tray • Automation PCR Adapter • QuantStudio™ 5 Strip Plate Tray • Thermo Scientific™ 96-Well PCR Base • Thermo Scientific™ PCR Strip Cutting Tool • Thermo Scientific™ PCR Capping Tool	A59905 A59019 A59018 A55332 A55330 A55329 A55327
Sample Enrichment Tubes	A59902
Enrichment Tubes Caps	A59903
Filter Tips for SureTect™ Automation 8/96[4]	A59033
Filter Tips for SureTect™ Automation 96[5]	A66298
Waste Bags for SureTect™ Automation	A59034
Protective Plate for SureTect™ Automation[5]	A66299
Additional materials	
Vortex mixer	PT0645 or equivalent

Table 7 Materials for automated lysis and qPCR sample preparation *(continued)*

Instrument and Appliances	Source
Automation Platform, one of the following:	
Plate centrifuge	MLS
Nunc™ Adhesive Seals (for cleaning the silicon mat)	236366

[1] Pipetting head can also be purchased separately (Automation CHOICE Head Package, Cat. No. A66296)
[2] Pipetting head can also be purchased separately (Automation Head R 96 Package, Cat. No. A66297)
[3] Purchase not required. A compatible laptop can be used.
[4] For use with the Automation Platform CHOICE Head and Automation Platform Head R 96.
[5] For use with Automation Platform Head R 96.

Materials for confirmation testing

Table 8 Materials for confirmation of positive results

Item	Source
Oxoid™ Campylobacter Blood-Free Selective Agar Base (Dehydrated), 500 g	CM0739B or equivalent
Oxoid™ CCDA Selective Supplement, 10 freeze-dried vials	SR0155E or equivalent
Remel™ Campy Cefex Agar	R110138 (USA)
Brilliance™ CampyCount Agar	R110168, PO1185A (Europe)
Oxoid™ O.B.I.S. campy Kit	ID0800M

AOAC *Performance Tested Methods*℠ validated workflow

Enrich food samples (page 17)

▼

Prepare the lysate using the thermal cycler method (page 19)

or

Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument (page 27)

▼

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v.3.0 or later (page 21)

or

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later (page 24)

▼ ▼

Confirm positive results (page 34)

2 Before you begin

Procedural guidelines

Guidelines for sample enrichment

- For preparation of initial suspensions, follow the instructions of EN ISO 6887 and EN ISO 16654 standards. Comply with Good Laboratory Practices (refer to EN ISO 7218:2007 standard).
- Follow the manufacturer's instructions for preparation of culture media.
- Use non-filtered homogenizer bags to help with fat and particle separation.
- For consistent PCR results, use a ventilated incubator.
- Follow the specified temperature allowances.
- Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

Guidelines for manual sample lysis

- Ensure that you use the recommended materials.
- For downstream PCR on the 7500 Fast instrument or the QuantStudio™ 5 Instrument —Prepare a mock-purified sample using sterile enrichment media as a negative extraction control. (The negative extraction control is required for RapidFinder™ Express Software; it is optional but recommended for RapidFinder™ Analysis Software.)

  Add the enriched sample or negative extraction control to the bottom of the lysis tube.
- For the thermal cycler method, in order to prevent crushing tubes, use one of the following:
  - Use the SimpliAmp™ Strip Plate Tray.

    Note: When the SimpliAmp™ Strip Plate Tray is used, the detachable side-edges of the 96-well lysis plate must always be broken off and discarded before placing the strips into the tray.

    Note: When running a full 96-well lysis plate in the SimpliAmp™ instrument, the SimpliAmp™ Strip Plate Tray is not required.

  or

  - Balance at least 4 complete tube strips in the sample block. It is recommended to space the strips evenly across the sample block, and if needed, add empty SureTect™ tubes to make 4 complete strips.

Guidelines for manual qPCR sample preparation

- Ensure that you use the recommended materials.
- PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
- **IMPORTANT!** After the lysate has been added to the pellets, vortex and spin the pellets and ensure that the pellets rehydrate immediately by tapping the tubes on the lab bench. Start the PCR run within 30 minutes.
- Tube and cap strips can be cut when less than a full strip is required.

  Do not cut the strips of caps or tubes too close to the wall of the tube or the cap lid, otherwise the lid might not seal adequately during PCR.
- After the PCR tubes have been opened, add lysate within 10 minutes.
- Particulate matter from the lysate can inhibit the PCR. To ensure that no particles are transferred from the Lysis Reagent 1 Tube to the PCR tube, remove lysate from the top half of the liquid, taking care not to disrupt the particles at the bottom of the tube.

  If the particles become disturbed, allow the particles to resettle for 1–2 minutes before lysate removal.
- Ensure that the pellet is fully dissolved. The solution changes from blue to green when the pellet is dissolved.
- For ease of use, a multi-channel pipettor can be used to transfer multiple lysates to the PCR tubes.
- Follow "Good laboratory practices for PCR" on page 41. For more information go to **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

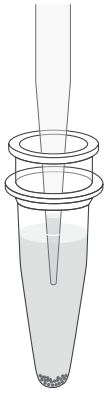

Figure 1 Avoid lysis particles

Guidelines for automated sample lysis and qPCR preparation

- Ensure that you use the recommended materials.
- Initiate the startup protocols for the QuantStudio™ 5 Food Safety Real-Time PCR Instrument and Automation Platform.
- For the automated workflow, the enrichments must be done using filter bags. Failure to use filter bags could lead to unsubstantiated results.
- The Sample Enrichment Tubes, or performing equivalent, must be used to enable correct pipetting height.
- The enriched samples must be placed in the Sample Enrichment Tubes in their specified rack.

  Note: The enrichment tubes must contain 700 µL–1000 µL of the enriched sample.
- The CyBio FeliX instrument is not compatible with pierceable seals.
- The Lysis Reagent 1 plate can be placed into its deck position with or without the Automation Lysis Tray. The Automation Lysis Tray is optional and not mentioned in the deck layout of the SureTect™ Automation Software.

- The caps of the sample tubes and lysis reagent tubes and the seals of the Lysis Reagent 1 Tubes must be removed before placing them on the deck of the Automation Platform.
- The PCR pellet plate must always be placed into the instrument with the QuantStudio™ 5 Strip Plate Tray and the Automation PCR Adapter for correct pipetting height.
- The seal of the PCR pellet plate must not be removed more than 10 minutes before the workflow is scheduled to start and before placing them on the deck of the Automation Platform.
- All accessories listed in the deck layout view of the software must be used.

Guidelines for spinning of PCR tubes

- SureTect™ workflow:
  - 20 µL of SureTect™ lysate is added to each PCR tube.
  - The capped PCR tubes are vortexed for 10–15 seconds to ensure that the pellet is fully rehydrated.
  - User must ensure that the reaction mixture is at the bottom of the PCR tube.
- A rapid spin-down is highly recommended before the PCR run to:
  - Collect the reaction mixture at the bottom of the well.
  - Remove bubbles.
- This ensures that the reaction conditions are optimal, and as a result, the PCR step is less likely to fail or to suffer from unwanted signal fluctuations which could affect the interpretation.
- Centrifugation of PCR tubes is included in every GLP (Good Laboratory Practice) protocol.

3 Enrich food samples

Enrich food samples

1. For all samples, pre-warm the indicated volume of media to 42±1°C.
2. Transfer the food sample to a non-filtered homogenizer bag, then add the media, as indicated.

Table 9 Enrichment conditions

Matrices	PCR instrument	Media	Incubation
Up to 325 g raw poultry product (ground poultry meat or raw poultry meat with skin, for example)	• QuantStudio™ 5 Food Safety Real-Time PCR Instrument and RapidFinder™ Analysis Software v3.0 or later • 7500 Fast Food Safety Real-Time PCR Instrument and RapidFinder™ Express Software v2.0 or later	325 g of sample and 1625 mL of Buffered Peptone Water. Mix thoroughly by hand massaging or stomaching for 10–30 seconds. Transfer 30 mL of the liquid portion to a new non-filtered sterile bag and add 220 mL pre-warmed Bolton Broth with Selective Supplement (no blood).	42±1°C for 22-48 hours
Up to 25 g processed poultry product (ready-to-reheat chicken nuggets, for example)		25 g of sample and 225 mL pre-warmed Bolton Broth with Selective Supplement (no blood) • For soft samples: homogenize for 10–30 seconds using a homogenizer. • For samples containing hard particles, such as bone: squeeze the bag by hand until the sample is mixed thoroughly with the media.	42±1°C for 22-30 hours
30 mL poultry carcass rinses		30 mL of sample and 220 mL pre-warmed Bolton Broth with Selective Supplement (no blood)	42±1°C for 22-48 hours
4 x 4" poultry carcass sponge		25 ml Buffered Peptone Water and 225 mL pre-warmed Bolton Broth with Selective Supplement (no blood)	42±1°C for 22-48 hours

Note: Enrichments can be conducted aerobically when there is minimal air remaining in the enrichment vessel, for example, with rolled enrichment bags or fit-for-purpose screw-cap containers.

3. Before incubation, exclude air by rolling down the tops of the enrichment bags to minimize the headspace of air contained in the bag. Use rolled bags with <5 mm of headspace after the sample is added.
4. Incubate as described in Table 9.
5. Remove the enriched sample from the incubator, briefly mix the liquid in the homogenizer bag/tube by hand, transfer an aliquot of sample from the side of the bag to a new tube, then close the tube and briefly mix.

   Retain sufficient sample for confirmation or repeat testing.

For manual lysis, proceed directly to Chapter 4, “Prepare the lysate”. For automated lysis and PCR sample preparation, proceed directly to Chapter 6, “Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument”. Or store the retained sample at 2–8°C for a maximum of 72 hours. Do not exceed 72 hours of total storage time.

4 Prepare the lysate

Prepare the lysate using the thermal cycler method

Before you begin, ensure that you are using the recommended materials.

Note: Always wear clean gloves when handling pierceable seals and store pierceable seals in the kit box or in an enclosed bag to prevent contamination of the pierceable seal.

1. Equilibrate the Lysis Reagent 1 Tubes to room temperature (23±5°C).
   a. Place the required number of Lysis Reagent 1 Tubes in a Thermo Scientific™ 96-Well PCR Base and SimpliAmp™ Strip Plate Tray. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.
   b. Ensure the liquid is collected at the bottom of each tube—flick the tubes in a downward motion or tap the tubes against the bench.
   c. Allow the tubes to remain at room temperature (23±5°C) for approximately 10 minutes before opening.
2. Remove the foil from each Lysis Reagent 1 Tube, then add 10 µL of Proteinase K to the tube.

   These tubes are referred to as Lysis Tubes in the rest of the procedure.

   IMPORTANT! Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL stepper pipette to reduce the number of tips required.
3. Transfer 10 µL of the enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a Lysis Tube.

   Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with Lysis Reagent 1.
4. Close or seal the tubes with the domed Lysis Tube Caps using the Thermo Scientific™ PCR Capping Tool or Thermo Scientific™ 96-Well Pierceable Seals using the MicroAmp™ Adhesive Film Applicator.

   Note: Samples sealed with domed Lysis Tube Caps and samples sealed with pierceable film can be placed together in the same run on the SimpliAmp.

   IMPORTANT! To prevent crushing the tubes in the SimpliAmp™ Thermal Cycler, use the SimpliAmp™ Strip Plate Tray, or use a full 96-well plate (see "Guidelines for sample lysis").

Note: Whenever the SimpliAmp™ Strip Plate Tray is used, the detachable side-edges of the 96-well lysis plate must always be broken off and discarded before placing the strips into the tray. There is no need to break off the side-edges of the strip plate if a full 96-well plate is used without the tray. When running a full 96-well lysis plate in the SimpliAmp instrument, the SimpliAmp™ Strip Plate Tray is not required.

Note: The SimpliAmp™ Strip Plate Tray Cover should not be used in the lysis step in the SimpliAmp™ Thermal Cycler.

5. Incubate the samples in the SimpliAmp™ Thermal Cycler using the following program settings.

Table 10 Program settings

Step	Temperature	Time
1	37°C	10 minutes
2	95°C	5 minutes
3	10°C	2 minutes
4	4°C	Hold[1]

[1] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

6. Ensure that the lid heater is on and set to 105°C, and the volume is set to Maximum.
7. Carefully remove the Lysis Tubes from the SimpliAmp™ Thermal Cycler. Do not agitate the Lysis Tubes.
8. Proceed to Chapter 5, “Perform PCR”.

(Optional) Store the samples at 2–8°C for up to 24 hours, including any time stored at 4°C in the thermal cycler.

Note: If pierceable seals have been used and already pierced, a second seal can be used for re-sealing the pierced tube before storing the lysate.

5 Perform PCR

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v.3.0 or later

This method is validated for lysates that are prepared using the thermal cycler method.

Before you begin, ensure that you are using the recommended materials.

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the **Help** function in the software for detailed instructions.

In the home screen of the RapidFinder™ Analysis Software, click **Create Experiment**, then enter or edit the well parameters.

Select **Campylobacter-Mpx-ST-A56835-QS5-1.1** or later for the assay.

Set up the PCR reactions

Before starting this procedure, see “Good laboratory practices for PCR” on page 41.

1. Following the plate layout previously set up in the software, place the required number of Campylobacter jejuni, C. coli and C. lari PCR Tubes in the QuantStudio™ 5 Strip Plate Tray. Place the tray on the Thermo Scientific™ 96-Well PCR Base. Ensure the PCR tubes sit firmly in the tray, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.

2. Allow the PCR tubes to remain on the bench for approximately 5 minutes, to bring to room temperature (23±5°C), then open one strip of PCR tubes by removing the seal.

   IMPORTANT! If all sample lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip of the PCR tubes, then proceed to the next step.

   - PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
   - If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.

3. If using domed caps, uncap the lysis tubes using the uncapping tool. If using a pierceable lysis seal, insert the SimpliAmp™ Strip Plate Tray Cover and pipette through the seal in the next step.

   Note: Make sure to clean the SimpliAmp™ Strip Plate Tray Cover after use to minimize the risk of contamination. Follow “Good laboratory practices for PCR” on page 41.

Note: Use of the SimpliAmp™ Strip Plate Tray Cover is optional.

4. Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet.

   IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube as this can inhibit PCR from occurring. Do not touch the pellet when adding the lysate.

5. Seal the PCR tubes with the flat optical PCR Caps provided with the kit.

   Ensure that the tubes are properly sealed by pressing down firmly over each opening. Use the PCR capping tool to seal the PCR tubes. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

6. If more than one strip of PCR tubes are required, repeat steps 2–5.

7. Mix all PCR tubes thoroughly by vortexing for 10–15 seconds to ensure that the pellet is fully rehydrated.

8. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. It is highly recommended to spin the PCR tubes for at least 10 seconds to remove the bubbles and to collect the liquid at the bottom of the tube before placing in the PCR instrument. Alternatively, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

1. Eject the instrument drawer. Use the QuantStudio™ 5 Strip Plate Tray to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.

2. In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.

3. Click **Start Run**, then follow the software prompts.

View results and data analysis

Data analysis is automated by the software. For detailed instructions and options for reporting, export, and storage of results, see the **Help** function in the software.

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- **Summary**—plate format
- **Results**—table format
- **Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning

PCR with the 7500 Fast Instrument and RapidFinder™ Express Software v2.0 or later

This method is validated for lysates that are prepared using the thermal cycler method.

Before you begin, ensure that you are using the recommended materials.

Set up the plate layout

RapidFinder™ Express Software determines the Run Layout (plate layout) for your samples based on the information entered and creates a run file. Refer to the Help function in the software for more details.

1. On the main page of RapidFinder™ Express Software, select **Create/Edit a Run File**, then enter or edit the Run File information at the prompts.
2. If desired, you can manually customize the plate layout.
3. If you modify the plate layout, you must rebalance the plate. The software will not do this automatically. To rebalance the plate, click the **Balance** button.
4. Select **Campybolacter_Multiplex_SureTect_7500_1.0** or later for the assay.

Set up the PCR reactions

Before starting this procedure, see “Good laboratory practices for PCR” on page 41.

1. Following the plate layout previously set up in the software, place the required number of Campylobacter jejuni, C. coli and C. lari PCR Tubes in the Thermo Scientific™ 96-Well PCR Base, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.

   If required by the plate layout, place empty low profile PCR tubes in the rack to balance the tray when the tubes are placed in the instrument.
2. Allow the PCR tubes to remain on the bench for approximately 5 minutes, to bring to room temperature (23±5°C), then open one strip of PCR tubes by removing the seal.

   IMPORTANT! If all sample lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip of the PCR tubes, then proceed to the next step.

   - PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
   - If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.
3. If using domed caps, uncap the lysis tubes using the uncapping tool. If using a pierceable lysis seal, insert the SimpliAmp™ Strip Plate Tray Cover and pipette through the seal in the next step.

   Note: Make sure to clean the SimpliAmp™ Strip Plate Tray Cover after use to minimize the risk of contamination. Follow “Good laboratory practices for PCR” on page 41.

   Note: Use of the SimpliAmp™ Strip Plate Tray Cover is optional.

4. Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet.

   IMPORTANT! Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

5. Seal the PCR tubes with the flat optical PCR Caps provided with the kit.

   Ensure that the tubes are properly sealed by pressing down firmly over each opening. Use the PCR capping tool to seal the PCR tubes. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.

6. If more than one strip of PCR tubes are required, repeat steps 2–5.

7. Mix all PCR tubes thoroughly by vortexing for 10–15 seconds to ensure that the pellet is fully rehydrated.

8. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. It is highly recommended to spin the PCR tubes for at least 10 seconds to remove the bubbles and to collect the liquid at the bottom of the tube before placing in the PCR instrument. Alternatively, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

In the RapidFinder™ Express Software, select **Start Instrument Run** on the main page, select the appropriate run file, and follow the software prompts.

1. Transfer the tubes to the 7500 Fast 96W Strip Plate Adaptor in the same configuration as the run layout.

   Note: The 7500 Fast 96W Strip Plate Adaptor is not compatible with single Armadillo tubes if the tube frame is broken off or removed.

   Be sure to load empty PCR tube strips as directed by the software (Figure 2).

2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.

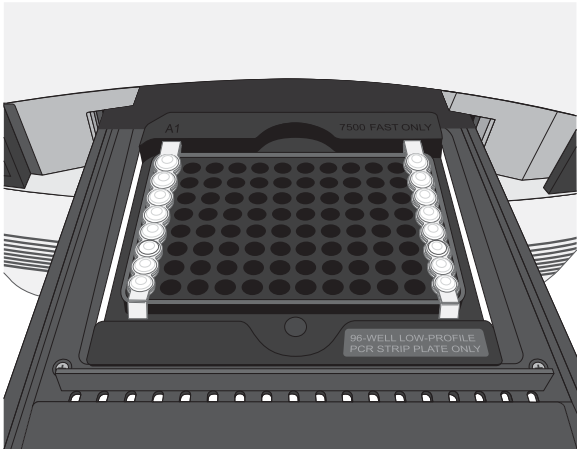

Figure 2 7500 Fast instrument tube layout

RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.

View results and data analysis

Data analysis is automated by the software.

In the RapidFinder™ Express Software, select **View Results** on the main page, select the appropriate run file, and follow the prompts to view results.

To display a list of results in table format, click **Table View**. Select a sample, then click **View Details** to see replicate information about samples.

RapidFinder™ Express Software results icons

Result icon[1]	Result
	Positive result
	Negative result
	Result warning

[1] RapidFinder™ Express displays results pictorially.

Options for reporting results

See the RapidFinder™ Express Software **Help** function for options to report, export, and store results.

6 Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument

Note: These instructions are for automated lysis and qPCR sample preparation using the automated workflow. For manual lysis instructions, see Chapter 4, “Prepare the lysate”.

Note: The instructions in this chapter are for the complete workflow. For all protocols there is the option to set up the run with step-by-step instructions (new users) or with a quick setup (experienced users). The instructions in this chapter are for the quick setup option. For a complete set of detailed instructions for all protocols, see the *SureTect Automation Workflow User Guide*.

Before you begin, ensure that you have read the “Guidelines for automated sample lysis and qPCR preparation” on page 15.

Complete quick setup protocol

Create the plate layout

Create the plate layout in the RapidFinder™ Analysis Software and export the file.

Select the protocol in the SureTect™ Automation Software

Select **Complete workflow**.

Choose the setup and define the plate layout

Select **Quick setup** and import the RapidFinder™ Analysis Software plate layout.

Prepare the materials on the laboratory bench

Place all materials except PCR plate on the instrument deck

Follow the instructions in the SureTect™ Automation Software to place the consumables in the assigned deck positions of the instrument.

Close the hatch to start the Lysis step

The lysis will be performed.

Continue with the PCR step or stop until a later time

You can stop after lysis if the PCR step will not be done immediately.

Load the PCR pellet plate

The SureTect™ Automation Software will prompt you to place the PCR plate into the instrument.

Close the hatch to start the PCR step

The PCR sample preparation will be performed.

Remove the PCR plate from the instrument

Manually seal, then vortex the PCR plate; spin, if needed

Start the qPCR run with the QuantStudio™ 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software

Create the plate layout

The plate layout for the automated workflow must be created in the QuantStudio™ 5 Real-Time PCR Instrument RapidFinder™ Analysis Software v3.0 or later. See the **Help** function in the software for detailed instructions.

The plate layout file is then exported (in .csv file format) and imported into the SureTect™ Automation Software which controls the Automation Platform.

Follow the steps to create the plate layout.

1. Open the RapidFinder™ Analysis Software.
2. Import the pathogen assay file.

   Assay files and instructions are available at **thermofisher.com/molecular-microbiology-software**.
3. Type in the Sample ID manually or use a barcode reader.
4. The plate layout must include an assay lot number. If using several boxes of the same lot in one run, they must have unique lot numbers for the instrument to be able to pipette the reagents from the correct tubes, for example,12345678box1, 12345678box2, etc.
5. Create the plate layout.
6. Export the *.csv plate layout file to a USB.
7. Open the SureTect™ Automation Software and import the plate layout file.

When using the Complete Workflow protocol, plate layouts for the sample tube rack, lysis plate, and PCR plate must be identical.

Prepare the materials for the complete workflow

Before you begin, equilibrate the Lysis Reagent Tubes and Campylobacter jejuni, C. coli and C. lari PCR Tubes to room temperature (23±5°C).

Follow the steps to prepare the materials for the automated procedure.

1. Prepare the Sample Enrichment Tubes
   a. Transfer between 700 µL to 1 mL of the enriched sample to each Sample Enrichment Tube.

b. The line in the Sample Enrichment Tubes is at the level of 800 µL and can be used as a reference when estimating the correct sample volume. See Figure 3.

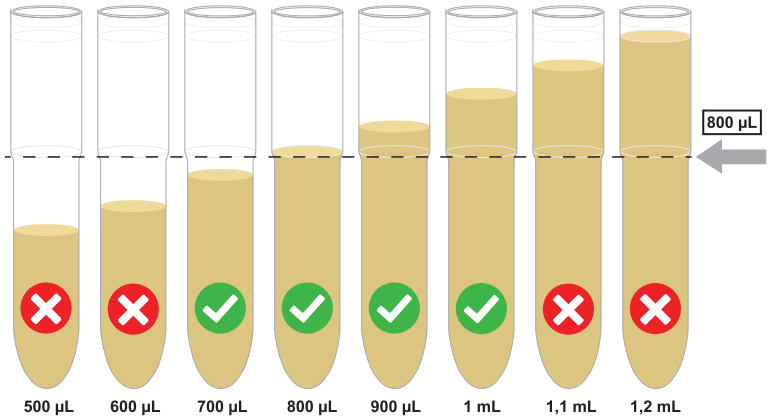

Figure 3 Sample Enrichment Tube level line

c. Prepare the Sample Enrichment Tubes in the tube rack according to the plate layout.

d. When prompted by the SureTect™ Automation Software, carefully place the Sample Enrichment Tubes with the tube rack into the instrument.

2. Prepare the Lysis tray on the laboratory bench.
   a. Place the Automation Lysis Tray (optional) on top of the laboratory bench.

   b. Place the required number of Lysis Reagent Tubes on the Automation Lysis Tray (optional) according to the imported plate layout. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.

   c. Ensure the liquid is collected at the bottom of each tube—flick the tubes in a downward motion or tap the tubes against the bench.

   d. Allow the tubes to remain at room temperature (23±5°C) for approximately 10 minutes.

   e. When prompted by the SureTect™ Automation Software, carefully remove the seals and place the Lysis Reagent Tubes (with or without the lysis tray) in the correct deck position according to the layout.

3. Prepare the PCR tray on the laboratory bench.

IMPORTANT! Do not open the Campylobacter jejuni, C. coli and C. lari PCR Tubes with PCR pellets until instructed to. The tubes must not be opened within a 10 minute time frame before loading them into the instrument.

   a. Place the QuantStudio™ 5 Strip Plate Tray on top of the Thermo Scientific™ 96-Well PCR Base on the laboratory bench.

   b. Place the Campylobacter jejuni, C. coli and C. lari PCR Tubes with PCR pellets on the QuantStudio™ 5 Strip Plate Tray according to the plate layout. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.

c. Ensure the PCR tubes sit firmly in the rack, then tap the tubes on the bench to ensure that the PCR pellets are located at the bottom of the tubes.

d. Allow the PCR tubes to remain on the bench for approximately 5 minutes to bring to room temperature (23±5°C).

e. After the lysis step is complete, you will be prompted by the SureTect™ Automation Software that it is time to load the PCR tubes for the PCR sample preparation step. At this point, you can carefully remove the seals.

f. PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.

g. If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip.

h. Place the QuantStudio™ 5 Strip Plate Tray with the opened PCR tubes on the Automation PCR Adapter according to the deck layout.

Run the SureTect™ Automation Software

Run the complete workflow

The complete workflow protocol includes the following automated steps:

- Dispensing the lysis reagents (Proteinase K and Lysis reagent 2) on the Lysis reagent 1 plate
- Dispensing the samples on the Lysis reagent 1 plate
- Lysis incubation step
- Dispensing the lysate on the PCR pellet plate

1. Power on the Automation Platform, then run the SureTect™ Automation Software.
2. At the **Home** screen, select **Complete workflow**.

   Note: Once the software is started, it will automatically detect which pipetting head is attached (CHOICE or 96).

3. In the **File import view** screen, select **Quick setup** and import the plate layout file created earlier. The **User ID** is optional.
4. In the **Lysis Reagents** screen, follow the directions to place the opened lysis reagents into the correct positions on the adapter.
5. In the **Deck layout** screen, follow the instructions to place the accessories and consumables in their assigned positions on the decks. Then, select the location of the next available tip in the TipBox on deck positions 2 and 4.
6. Close the hatch before starting the run.

7. Select **Yes** to start the lysis.

   Note: Once the run is started, the screen will show a timer that displays the time remaining until the lysis step is complete.

8. In the next **Deck layout** screen, follow the instructions to place the opened PCR tubes onto the deck.

9. Close the hatch to start the run.

10. Select **Yes** to start the PCR setup.

    Note: Once the run is started, the screen will show a timer that displays the time remaining until the PCR step is complete.

11. When complete, remove the PCR tubes, then proceed to seal, vortex, and spin, if needed.

12. Start the PCR run with the QuantStudio™ 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software, using the plate layout created for this sample set.

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v3.0 or later

Before you begin, ensure that you are using the recommended materials.

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the **Help** function in the software for detailed instructions.

In the home screen of the RapidFinder™ Analysis Software, click **Create Experiment**, then select the plate layout previously created.

Select **Campylobacter-Mpx-ST-A56835-QS5-1.1** or later for the assay.

Load and run the reactions

1. Eject the instrument drawer. Use the QuantStudio™ 5 Strip Plate Tray to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.

2. In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.

3. Click **Start Run**, then follow the software prompts.

View results and data analysis

Data analysis is automated by the software. For detailed instructions and options for reporting, export, and storage of results, see the **Help** function in the software.

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- **Summary**—plate format
- **Results**—table format
- **Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning

7 Confirm positive results

Recommended confirmation methods

Samples with positive PCR results must be confirmed by one of the following tests.

- Perform selective plating and the Oxoid™ O.B.I.S. campy Kit test (see “Isolate presumptive positives” on page 34).
- Depending on the legislation territory, using conventional tests described in the methods standardized by CEN or ISO, or any appropriate national standard (e.g. USDA/FSIS) including the purification step. It is as well possible to use an appropriate ISO 16140-6:2016 or AOAC-OMA validated method. The confirmation step must start from the primary enrichment broth.

In the event of discordant results (presumptive positive with the alternative method, not confirmed by one of the means described above/below and in particular by the Oxoid™ O.B.I.S campy Kit test), the laboratory must employ adequate means to ensure the validity of the result obtained.

Isolate presumptive positives

1. Streak 10 µL of enriched sample onto mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar.
2. Incubate at 42±1°C for 48 hours under microaerophilic conditions.
3. Confirm presumptive positive samples using reference method techniques (EN ISO 10272-1:2017 or USDA FSIS MLG 41.04, for example), or the Oxoid™ O.B.I.S. campy Kit, or an appropriate ISO 16140-6:2016 or AOAC-OMA validated method depending on the legislation territory.

Confirmation in case of co-infection

The SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay uses a multiplex PCR reaction to detect multiple *Campylobacter* species in a single reaction tube. In cases of co-infection, where more than one species is present in the same sample, confirmation testing for each individual species may be a challenge as these organisms are not differentiated on plating media. This is especially challenging when one species comprises the major proportion of the population and another species comprises the minority of the population.

The likelihood of isolation for species found in the minority of the population can be estimated by reviewing PCR amplification plots and determining the difference in C_t value between the species that represent the major and minor populations. If this difference is large (for example, C_t >3.0) the likelihood of isolating and confirming the minor population is lowered. In these cases, users may experience confirmation of the major population but not the minor.

8 Confirm presumptive colonies using SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay - EN ISO 7218:2007

Confirm characteristic colonies using SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay

The ISO 7218:2007 standard outlines the use of nucleic acid probes to confirm presumptive characteristic colonies if validation data is available [e.g. bibliography, ISO 16140, and/or AOAC validation study(ies)]. Therefore, the SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay can be used for presumptive colony confirmation.

However, good laboratory practice suggests using different methods to screen and confirm samples. Adhering to this guidance, the SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay should be used either for detection purposes or confirmation of characteristic colonies recovered on selective agar plates.

When using the SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay to confirm colonies, the sample preparation is as follows:

1. Emulsify an isolated colony in 1 mL of saline.
2. Use this as a test sample by adding 10 µL input and follow the protocol described in Prepare the lysate (page 19).
3. Perform PCR as described in Perform PCR (page 21).

Note: It is recommended that users perform implementation verification as described in ISO 16140-3:2021.

A

Troubleshooting

Observation	Possible cause	Recommended action
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal is detected, but a target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred. Additionally, a problem with the IPC occurred due to: • Preferential amplification of the carryover DNA. • Carryover of particles from the Lysis Tube.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal or an exceptionally weak or atypical IPC amplification plot is detected. The result is considered invalid by the software.	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.
	Incomplete lysis steps caused an inhibition of the PCR.	Retest the original sample and diluted sample, ensuring that the correct heating parameters are followed.
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software.	Inhibition of PCR occurred, due to: • Carryover of particles from the Lysis Tube. • PCR inhibitors present in the food sample. • Incomplete sample lysis. • Other, unknown, cause.	Retest the original sample and its dilution. To remove the impact of PCR inhibitors in the sample, dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media), or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.

Observation	Possible cause	Recommended action
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software. *(continued)*	Bubbles were present in the PCR tube.	Inspect each tube for bubbles by looking through the optical PCR Caps. Large bubbles can often be removed by firmly holding the top of the tube while gently flicking the bottom. If the bubble persists, spin the tube for 10 seconds in a plate spinner. If the bubble continues to persist, set up a new PCR tube using the prepared lysate.
In test samples, no IPC signal is detected, but target-specific signal is detected. The result is considered invalid by the software.	A problem occurred in IPC amplification due to preferential amplification of the target-specific DNA.	Retest the original sample and diluted sample. Dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media) or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.
In test samples that are expected to be positive, no target-specific signal is detected.	Certain sample types contained components that were inhibitory to the growth of the target organism.	Dilute the sample pre-enrichment to a level that prevents growth inhibition of target bacteria. For example, following guidelines in ISO 6887.
In confirmation testing, suspect colonies on mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar are not present	Overgrowth of *Campylobacter* with background flora occurred.	Sub-culture 1 mL of the retained enrichment into 9 mL of Bolton Broth with blood. Incubate at 41.5 °C for 40–48 hours under microaerophilic conditions. Streak onto mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar and incubate at 41.5 °C for 40–48 hours under microaerophilic conditions. Continue with the confirmation.
	DNA from non-viable cells caused an unconfirmed positive result.	Sub-culture 1 mL of the retained enrichment into 9 mL of Bolton Broth with blood. Incubate at 41.5 °C for 40–48 hours under microaerophilic conditions. Streak onto mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar and incubate at 41.5 °C for 40–48 hours under microaerophilic conditions. Continue with the confirmation.
In confirmation testing, suspect colonies on mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar are too small to conduct confirmation tests	The isolate was sensitive to selective components in the medium or the lower limit of the incubation time was used.	Purify the well-isolated, suspect colony on a non-selective plating medium to increase biomass before continuing with confirmation.
In confirmation testing, suspect colonies on mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar are not well isolated	The enriched sample contained high levels of background flora that were not inhibited on mCCD agar, Remel™ Campy Cefex Agar, or. *Brilliance*™ CampyCount Agar	Purify the suspect colonies on a second mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar plate before continuing with confirmation.

Observation	Possible cause	Recommended action
In confirmation testing, presumptive PCR positives are not confirmed on chosen selective media (mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar)	The isolate was sensitive to selective components in the medium, the organism was stressed and struggled to recover on the particular plate, or the lower limit of the incubation was used.	Re-streak the enrichment onto a selective mCCD agar, Remel™ Campy Cefex Agar, or *Brilliance*™ CampyCount Agar plate before continuing with confirmation.

Test control organisms

Incorporation of positive control organisms is not necessary with routine testing of samples, because the PCR results are validated if the IPC signal is detected. However, you may choose to use target isolates to ensure that the workflow, an assay, and/or a batch performs as it should.

If testing of positive control organisms is required, select a suitable organism recommended by Thermo Fisher Scientific, Microbiology Division. Contact your local supplier for further information.

Process a control organism in parallel with test samples through sample enrichment, lysis, and PCR, following your laboratory methodology.

The following instructions were generated to mitigate the risk of laboratory cross-contamination when handling target strains and provide general guidelines for positive control preparation.

IMPORTANT! Aseptic techniques and sterile consumables should be used at all the times.

- Strain selection and culture:
  - Select a suitable organism.
  - Streak the isolate onto an agar plate to obtain isolated colonies.
  - Incubate the plate under suitable conditions until colonies are visible easily by the naked eye.
- Sample preparation:
  - Pick a single well-isolated colony using a suitable sterile tool (e.g., pipette tip or culture loop).
  - Emulsify the colony carefully in 1 mL of saline or sterile enrichment broth.
  - Dilute the initial suspension using the same medium to obtain C_t value ~ 25-30 (e.g. 1:10 or 1:50).
  - Prepare lysate using 10 µL of diluted suspension instead of enriched sample. When possible, it is recommended to add the positive sample lysate on the PCR plate only after the (unknown) sample tubes have already been sealed.
  - Run PCR according to standard procedure.

    Note: Do not open the PCR tubes after the PCR run has completed.

RapidFinder™ Express Software results warnings

RapidFinder™ Express Software v2.0 may indicate a result warning due to inhibition for some samples. In some rare cases the warning label is result of **Non-linear baseline** notification for the bacterial targets and/or IPC detector of the assay.

In such rare cases, follow the recommended workflow:

1. Select **View details** to manually view results of the highlighted reaction for the bacterial targets and the IPC in the RapidFinder™ Express Software v2.0.
2. Inspect the IPC result.
3. Inspect the bacterial target results.

If the C_t of the IPC is below the cut off C_t value depicted in following table and the bacterial targets have received a negative interpretation and the signal is above the cut off C_t value, the result can be interpreted as true negative.

Whenever the IPC and bacterial targets have received C_t values below the cut off C_t values depicted in the following table , proceed to a confirmation step as described in the user guide.

In case of a negative IPC result or IPC C_t above the cut off, follow the instructions given in the user guide to repeat the sample.

Assay	Cut off for target C_t value	Cut off for IPC C_t value
SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay	40 for each target	36

B

Supplemental information

AOAC *Performance Tested Methods*℠ Certification

Table 11 *Performance Tested Methods*℠ Certification of the workflow

Certification
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 012101

The detection of *Campylobacter jejuni, C. coli,* and *C. lari* using the SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay has earned the AOAC *Performance Tested Methods*℠ Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment as described in "Enrich food samples" on page 17
- SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 12 for characteristics) with RapidFinder™ Analysis Software v3.0 or later and Pathogen Assay File: **Campylobacter-Mpx-ST-A56835-QS5-1.1** or later

Table 12 QuantStudio™ 5 Real-Time PCR Instrument characteristics

Characteristics	QuantStudio™ 5 Real-Time PCR Instrument
Optics	Bright white LED
Filters	6 excitation and 6 emission filters
Sample ramp rate	Average: 3.66°C/sec Maximum: 9.0°C/sec
Thermal range	4–99°C
Thermal accuracy	±0.25°C
Thermal uniformity	±0.4°C
Format	96-well, 0.1-mL block

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 13 for characteristics) with RapidFinder™ Express Software v2.0 or later and Pathogen Assay File: **Campybolacter_Multiplex_SureTect_7500_1.0** or later

Table 13 7500 Fast Real-Time PCR Instrument characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: ±1.6°C/sec Fast mode: ±3.5°C/sec
Thermal range	4–100°C
Thermal accuracy	±0.5°C
Thermal uniformity	±1°C
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples using mCCD agar or Remel™ Campy Cefex Agar, as described in "Recommended confirmation methods" on page 34

Table 14 Validated matrices

Matrices
325 g ground poultry
325 g poultry with skin
25 g ready-to-reheat processed poultry
30 mL carcass rinses
4 x 4" poultry carcass sponge

Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.

- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2005 or **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

Symbol definitions

Symbol	Definition
	BATCH CODE
	CATALOGUE NUMBER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE
	MANUFACTURER
	TEMPERATURE LIMIT (storage temperature)
	USE-BY DATE

C Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
  www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 4th Edition, WHO/CDS/CSR/LYO/2020.12; found at:
  www.who.int/publications/i/item/9789240011311

D Documentation and support

Food safety support

Website: **https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html** or **thermofisher.com/foodsafety**

Support email:

- Europe, Middle East, Africa: **microbiology.techsupport.uk@thermofisher.com**
- North America: **microbiology@thermofisher.com**

Phone: Visit **thermofisher.com/support**, select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Related documentation

All of the SureTect™ IFUs are located at **www.thermofisher.com/suretect-ifu**.

Document	Publication number
CyBio FeliX Operating Instructions	7062-B
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
SimpliAmp™ Thermal Cycler User Guide	MAN0009889
SimpliAmp™ Thermal Cycler Installation and Operation Quick Reference	A24827
RapidFinder™ Express Software Quick Reference	4480999

References

EN ISO 10272-1:2017. Microbiology of food the food chain – Horizontal method for the detection and enumeration of *Campylobacter* spp – Part 1: Detection method.

EN ISO 6887-1:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

EN ISO 6887-2:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 2: Specific rules for the preparation of meat and meat products.

EN ISO 6887-3:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 3: Specific rules for the preparation of fish and fishery products.

EN ISO 6887-4:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of miscellaneous products.

EN ISO 6887-5:2010. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products.

EN ISO 7218:2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.

EN ISO 22174:2005. Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definition.

EN ISO 16140-6:2019. Microbiology of food and animal feed – Method validation – Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

EN ISO 16140-3:2021. Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory.