Pathatrix™ Salmonella spp. Pooling Kits Linked to MicroSEQ™ Salmonella spp. Detection Kit

USER GUIDE

For food samples

for use with:

Pathatrix™ 5 Pooling Salmonella spp. Kit
Pathatrix™ 10 Pooling Salmonella spp. Kit
Pathatrix™ Auto Instrument
MicroSEQ™ Salmonella spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR System
RapidFinder™ Express Software v2.0 or later

Catalog Numbers APS250P, APS500P, 4403930
Publication Number MAN0006977
Revision C.0

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 090203C

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

Revision history: Pub. No. MAN0006977

Revision	Date	Description
C.0	13 March 2023	• Instructions were removed for enrichment of 375 g cocoa and chocolate-based samples. • Instructions were changed from 375 g of sample to 325 g of sample.
B.0	11 November 2021	• The Pathatrix™ 5-Pooling Kit was added. • Instructions were added for enrichment of 375 g cocoa and chocolate-based samples. • The software version was updated for RapidFinder™ Express Software. • Characteristics of the 7500 Fast Real-Time PCR Instrument were added. • The user guide template was updated with associated updates to the general document organization, limited license information, trademark statement, safety statements, and support information.
A.0	1 February 2013	New document created for the Pathatrix™ Salmonella spp. Pooling Kits Linked to MicroSEQ™ Salmonella spp. Detection Kit.

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.

Overview

The Pathatrix™ Salmonella spp. Pooling Kits are sample preparation methods for presence/absence testing based on the detection of as few as 1–10 cfu (colony forming units) per 25–325 g of food sample.

Using the Pathatrix™ 10-Pooling or 5-Pooling Salmonella spp. Pooling Kit linked to the MicroSEQ™ Salmonella spp. Detection Kit, presumptive results can be obtained, prior to confirmation, in as little as 20 hours. A presumptive positive isolate should be subsequently confirmed by the use of subculture, as well as appropriate biochemical and serological tests as required.

Once confirmed, the results are reported as:

- *Salmonella* spp. Detected in 25–325 g (sample matrices)
- *Salmonella* spp. Not detected in 25–325 g (sample matrices)

This guide describes an AOAC *Performance Tested Methods*℠ workflow for detection of *Salmonella* spp. in food samples (see Appendix C, "AOAC *Performance Tested Methods*℠ Certification" for details).

Before starting this workflow, ensure that the instruments are properly installed and calibrated. For calibration information, see the documentation that is provided with the instruments.

Contents and storage

Table 1 Pathatrix™ 10 Pooling Salmonella spp. Kit (Cat. No. APS500P; 50 cartridge runs; 50 pools of 10 samples; 500 samples) and Pathatrix™ 5 Pooling Salmonella spp. Kit (Cat. No. APS250P; 50 cartridge runs; 50 pools of 5 samples; 250 samples)

Item	Amount	Storage
• Pre-sterilized Sample and Elution Vessel Packs • Pre-sterilized Capture Phase Packs • Pre-sterilized Flat Cap Lids	50 each[1]	Room temperature
Anti-*Salmonella* spp. Antibody-Coated Paramagnetic Beads[2]	2.5 mL	5±3°C[3] **Do not freeze.**[4]

[1] The Pathatrix™ Auto Plastics Kit is also available as Cat. No. ZNACPT
[2] The beads are available separately as Cat. No. ZBSQCAP500.
[3] Refer to the product label for the expiration date.
[4] Beads that have been subjected to freezing temperatures may be rendered inactive.

Table 2 MicroSEQ™ Salmonella spp. Detection Kit (96 reactions, Cat. No. 4403930)

Contents	Amount	Cap color	Storage[1]
Salmonella spp. Assay Beads, 8-tube strips	12 strips (96 tubes) 1 rack	Green (rack)	5±3°C Protect from light and moisture.[2]
MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	N/A	
Pathogen Detection Negative Control[3]	1.5 mL	Red	5±3°C

[1] See the product label for the expiration date.
[2] Excessive exposure to light may affect the fluorescent probes. To protect the beads from moisture, do not remove the desiccant from the pouch, and seal the pouch tightly each time you remove assay bead strips.
[3] The Pathogen Detection Negative Control is included in a separate box and may be shipped separately.

Required materials

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

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

Materials for immunomagnetic separation

Table 3 Required materials not included in the Pathatrix™ kits

Item	Source
Pathatrix™ Auto Instrument, including Sample Vessel Holder[1] and Elution Vessel Holder[1]	PTXAUTOINS
(Optional) Pathatrix™ Cartridge Rack; holds 5 Cartridges	ACARTRACK
PBS, 10X, pH 7.4 Dilute 1:10 in molecular biology-grade water (Cat. No. AM9932 or equivalent) prior to use.	AM9624 or AM9625
Nuclease-free water	AM9932 or equivalent
Optional, for pooling high-particulate or high-fat-content samples:	
Pathatrix™ Foam Filters	PFF
Pathatrix™ 5 Pooling Kit [contains Straws (254 mm) and Syringes (10 mL)]	POOL510MLN
Pathatrix™ 10 Pooling Kit [contains Straws (254 mm) and Syringes (10 mL)]	POOL1010MLN

[1] Available separately as Cat. No. ATUBERACK.

Materials required for sample enrichment

Table 4 Materials for food samples

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

Table 4 Materials for food samples *(continued)*

Item	Source
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, 37±1°C	MLS
Enrichment media appropriate for the sample type:	
Buffered peptone water (BPW)	DF1049, CM1211B, or equivalent
Brilliant Green (CAS 633-03-4)	Fisher Scientific™ B422-25 or equivalent
Ultra-high temperature (UHT) processed skim milk or Non Fat Dried Milk (NFDM)	Food retail store

Table 5 Materials for environmental samples

Item	Source
Swabs or sponges	MLS
Dey-Engley Neutralizing Broth, for prewetting swabs or sponges	R060770, R09334 (tubes), R112160 (WMB lug), or MLS
Sterile 15-mL tubes, for use with swabs	MLS

Materials for PCR-ready DNA sample preparation

Table 6 Materials for PCR-ready DNA sample preparation

Item	Source
Heating block, 97±2°C	MLS
DynaMag™-2 Magnet (for use with microcentrifuge tubes)	12321D
Sterile 1.5-mL microcentrifuge tubes	MLS
Nuclease-free water	AM9938 or equivalent
Lysis Buffer, FS	4480724
(Optional) **For processing large numbers of samples in a 96-well plate format**	
(Optional) Thermal cycler	SimpliAmp™ Thermal Cycler
(Optional) 96-well PCR plates and non-optical film	4346906 and 4306311, or equivalent

Table 6 Materials for PCR-ready DNA sample preparation *(continued)*

Item	Source
(Optional) Magnetic rack; for example, Pathatrix™ Magnetic Capture Plate or Magnetic Stand-96	One of the following or equivalent: • MAGNETICPLATE • AM10027

Materials for PCR detection

Item	Source
Instruments and equipment	
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative.
RapidFinder™ Express Software v2.0 or later	Download the latest version at **thermofisher.com/rapidfinder-express-software**
7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips	A29252
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Cap Installing Tool	4330015
MicroAmp™ Multi-removal Tool	4313950
Benchtop microcentrifuge with 8-tube strip adapter *or* Plate centrifuge	MLS
Laboratory mixer (vortex mixer or equivalent)	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp™ Fast 8-Tube Strip, 0.1-mL[1]	4358293
MicroAmp™ Optical 8-Cap Strip, 300 strips[1]	4323032

Item	Source
Reagents	
Nuclease-free water	AM9938

[1] Required to evenly distribute the clamping load applied to the tube strips during PCR processing. Do not use other tube strips, which could result in crushed tubes.

Materials for confirmation testing of PCR-positive samples

Table 7 Materials for confirmation testing of PCR-positive samples

Item	Source
Incubator, 37±1°C	MLS
Sterile, disposable 10-µL loops	MLS
Xylose lysine deoxycholate (XLD) agar	CM0469R, CM0469B, or equivalent
Second selective agar, for example:	
Modified Brilliant Green Agar	CM0329B or equivalent
Brilliance™ Salmonella Agar[1]	• CM1092B (base) • SR0194E (supplement)

[1] Contact your Thermo Scientific Microbiology sales representative for prepared media options.

Workflow

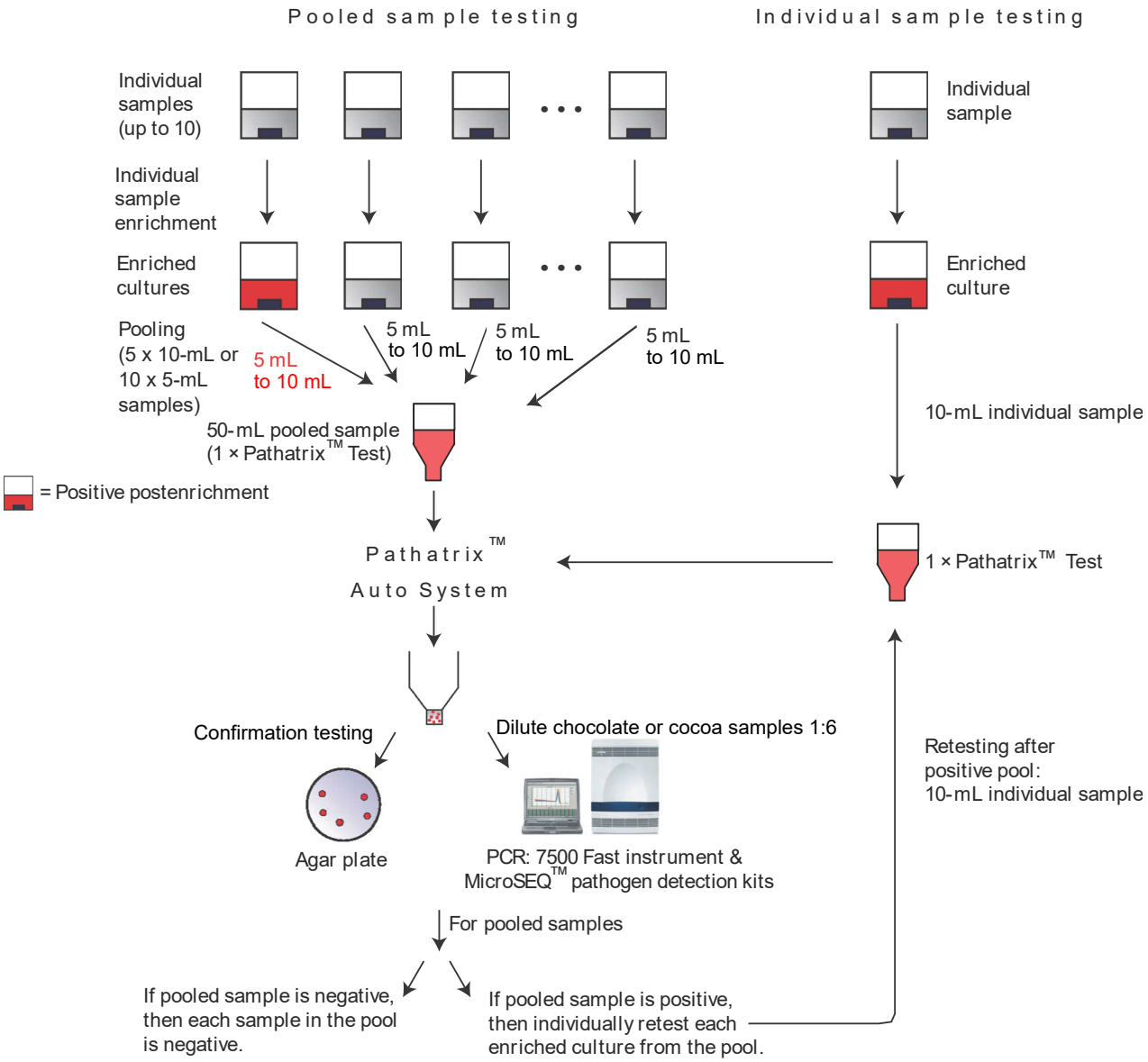


Figure 1 Workflow

2 Enrich samples

Procedural guidelines

- Use aseptic technique and good laboratory practices at all times.
- Wear a facemask when weighing out powders.
- Take care when boiling agar before autoclaving. Wear heat-resistant gloves when handling hot flasks of liquid.
- Take care when handling vessels that contain microorganisms.
- Avoid generating aerosols, as pathogenic organisms may be present.
- Dispose of used or unused reagents, used media, sample enrichments, as well as other contaminated disposable materials following procedures for infectious or potentially infectious products.
- Treat all waste as biohazardous, and handle and dispose of using safe laboratory practices, in accordance and compliance with all appropriate regulations.

Enrich food or environmental samples

Note: See Appendix C, "AOAC *Performance Tested Methods*℠ Certification" for details about matrices used in the AOAC validation study.

Certain food types (for example, dairy and chocolate) and swabs/sponges benefit from an alternative enrichment strategy. See Appendix B, "Alternative enrichment methods" for alternative sample preparation and enrichment recommendations for dairy, chocolate, and acidic/alkaline samples.

1. Prepare the sample for enrichment according to the following table.

Sample type	Preparation method
Food samples	1. Prepare a 1 in 10 dilution of the food sample in pre-warmed Buffered Peptone Water (BPW) pre-warmed to 34–38°C in a sterile homogenizer bag with an internal strainer. For example: • Add 25 g of food sample to 225 mL of BPW • Add 375 g of food sample to 3375 mL of BPW **Note:** A standard sterile homogenizer bag or equivalent can be used with liquid samples. **Note:** For cocoa and chocolate, ensure the addition to the enrichment broth of the following: • Brilliant Green (0.018 g/L) for products with high background flora • Tween™ 80 for the products with >20% fat content 2. Homogenize the sample for approximately 1 minute at normal speed in a homogenizer or laboratory blender. Alternatively, hand mix for approximately 1 minute, massaging the sample between the fingers to disperse any large clumps of material (Narang and Cray, 2006).
Environmental contact swabs[1]	1. Wipe an area with a prewetted swab. **Note:** Rehydrate swabs with approximately 0.5 mL of Dey-Engley Neutralizing Broth, or equivalent. 2. Transfer the swab to a sterile 15-mL tube containing 10 mL of Buffered Peptone Water prewarmed to 34–38°C, then twirl in the broth for about 60 seconds.
Environmental contact sponges[1]	1. Wipe an area with a prewetted sponge. **Note:** Rehydrate sponges with approximately 10 mL of Dey-Engley Neutralizing Broth, or equivalent. 2. Transfer the sponge to a sterile homogenizer bag containing 100 mL of Buffered Peptone Water prewarmed to 34–38°C.

Sample type	Preparation method
	3. Homogenize the sample for approximately 1 minute at normal speed in a homogenizer or laboratory blender. Alternatively, hand mix for approximately 1 minute, massaging the sample between the fingers.

[1] Not included in the AOAC validation study.

2. Incubate at 34–38°C for 20±2 hours, or 20-28 hours for cocoa and chocolate products enriched in UHT skimmed milk or NFDM, or 22-30 hours for cocoa and chocolate products enriched in BPW.

Proceed to "Transfer the enriched culture to the Sample Vessel" on page 16 immediately (recommended).

If storage of sub-samples is required, store at 5±3°C for up to 32 hours. Rewarm samples to 34–38°C before analysis on the Pathatrix™ Auto Instrument.

Store the remaining enriched sample at 5±3°C for up to 32 hours, for potential reanalysis.

3 IMS capture of *Salmonella* spp. with the Pathatrix™ beads

Workflow

Transfer enriched culture to the Sample Vessel

(Pool up to 10 samples of enriched cultures at this step)

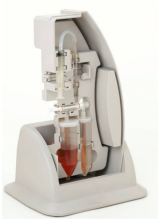
▼

Load and run the samples

▼

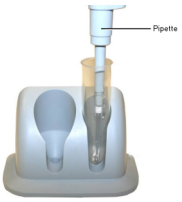
Unload the samples

▼



Capture the Pathatrix™ beads

▼



Proceed to PCR

Transfer the enriched culture to the Sample Vessel

1. Remove the Sample and Elution Vessels from the Pathatrix™ consumable kit packaging and place into the Sample Vessel Holder.
2. Partially remove the lids from both vessels, making a large enough opening to allow the addition of sample and wash buffer to the vessels.
3. Prepare a single pooled sample by transferring between 5-mL and 10-mL aliquots from up to ten (10) individually enriched samples to the Sample Vessel. Total pooled sample should equal 50 mL.

Note: If the samples are highly particulate and/or contain a high fat content, we recommend using foam filters with pooling syringes and straws.

Store the individual enriched samples at 5±3°C for potential reanalysis until the test result is confirmed.

Note: Do not store enriched samples for more than 32 hours.

Load and run the sample

1. Add about 35 mL of 1X PBS to the fill line of the Elution Vessel.
2. Replace the lids on the Sample and Elution Vessels, making sure that the vessels are completely sealed.
3. Add 50 µL of the Pathatrix™ bead suspension into the spout on the lid of the Sample Vessel.

   IMPORTANT! Fully resuspend the Pathatrix™ beads by agitating the bead vial (for example, by vortexing or inversion of sealed bead vial) before adding to the Sample Vessel.

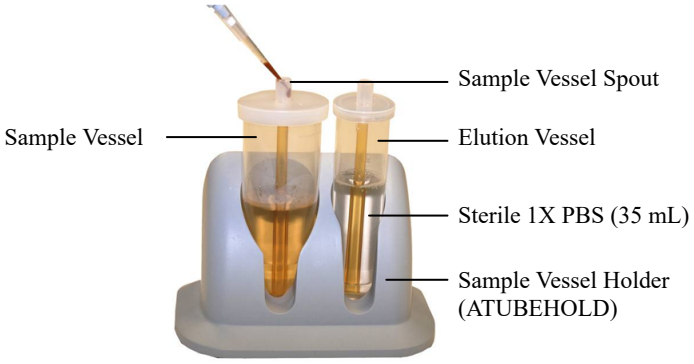


Figure 2 Add Pathatrix™ beads to the Sample Vessel

4. Orient the capture phase kit with the valve plunger pointing left and connect the valve firmly to the lids of the Sample and Elution Vessels.

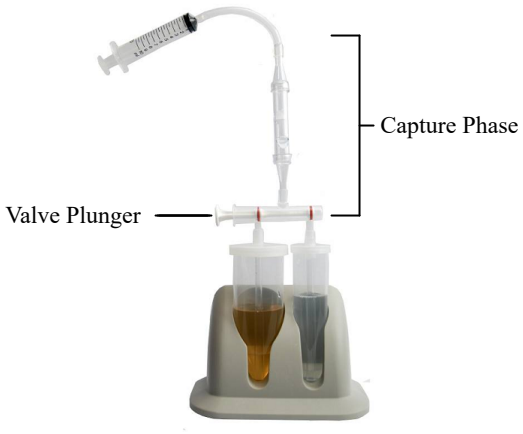


Figure 3 Sample and Elution Vessels assembled with capture phase

While handling the assembly, be careful not to rotate, twist, or bend the conical ends of the capture phase, as this may loosen or separate the assembly and create leaks that will affect performance.

5. Holding both vessels, carefully lift the assembled vessels and attached capture phase kit out of the Sample Vessel Holder (Figure 3).

6. Place the vessels into the Cartridge, pushing the vessels firmly in place from the bottom upwards.

7. Firmly push the remainder of the kit into the Cartridge, ensuring that the valve, phase, and syringe are all held securely in the molded recess of the Cartridge (Figure 4).

8. Push the magnet slider across into the locking position and press the magnet release button on the side of the Cartridge to ensure the kit is positioned correctly and the magnets can freely disengage from the phase.

IMPORTANT! If the magnet slider is hard to close or does not release cleanly when the release button is pressed, carefully reposition the capture phase and repeat this step. Failure to ensure smooth release will cause magnetic beads to remain captured and reduce overall performance.

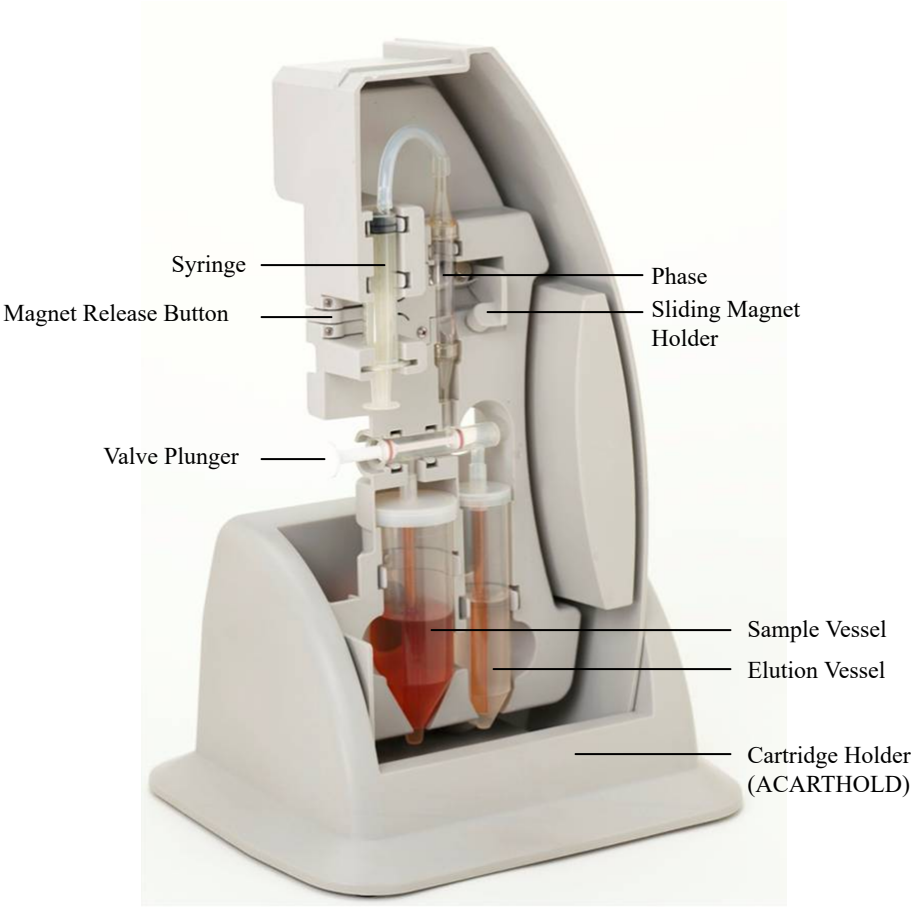


Figure 4 Assembled Cartridge

9. Reset the magnets into the locked position.

10. Insert the Cartridge into the Pathatrix™ Auto Instrument until it clicks into position.

11. Press the numbered button above the appropriate Cartridge to start the run.
The associated LED turns green to indicate the run has started.

After ~12 minutes, the LED flashes red and green alternately, prompting the user to unload the sample, as described in the next section.

Unload the sample

1. Press the button above the appropriate Cartridge to initiate the draining step (approximately 2 minutes).
2. When the draining step is complete and the LED is illuminated red, remove the Cartridge by pulling it out, away from the instrument.
3. Remove the syringe from the Cartridge and carefully pull out the rest of the kit, starting at the top and working downwards.

   When removing the Sample and Elution Vessels from the Cartridge, hold firmly by the vessels themselves to prevent spillage.
4. Place both vessels with the capture phase attached in the Sample Vessel Holder.
5. Remove the lid from the Elution Vessel to separate it from the rest of the consumable.
6. Leaving the Elution Vessel in the Sample Vessel Holder, lift away and discard the rest of the consumable, including the Sample Vessel.

Note: The Elution Vessel contains approximately 200 µL of PBS and Pathatrix™ beads with the captured bacteria.

Capture the Pathatrix™ beads

In this procedure, the Pathatrix™ beads are magnetically captured and washed using a Sample Vessel Holder (holds 1 Elution Vessel) or an Elution Vessel Holder (holds up to 5 Elution Vessels). Both holders contain magnets to capture beads along one side of the bottom of the Elution Vessels.

1. Leave the Elution Vessel in the Sample Vessel Holder for approximately 1 minute to allow capture of the Pathatrix™ beads.
2. **For chocolate and cocoa-based samples only:** after capture of the beads in step 1, pipet the supernatant up and down very gently, to resuspend any cocoa sediment, without disturbing the captured beads.

   This step optimizes removal of chocolate-matrix residue.
3. While the Elution Vessel is still in the Sample Vessel Holder, carefully remove and discard the PBS from the Elution Vessel, taking care not to disturb the captured Pathatrix™ beads.
4. *(Optional; for sample types that carry over large amounts of debris into the Elution Vessel)* If, when removing the PBS from the Elution Vessel, you notice that the solution is not clear, perform a second, manual PBS wash on the beads to prevent lysis and PCR inhibition.
   a. Remove the Elution Vessel from the Sample Vessel Holder, add 120 µL of 1X PBS, and pipet up and down to completely resuspend the Pathatrix™ beads.
   b. Place the Elution Vessel in the Sample Vessel Holder and allow it to remain there for approximately 1 minute.

c. While the Elution Vessel is still in the Sample Vessel Holder, carefully remove and discard the PBS without disturbing the captured Pathatrix™ beads.

5. Remove the Elution Vessel from the Sample Vessel Holder and completely resuspend the Pathatrix™ beads in 120 µL of nuclease-free water by pipetting up and down.

4 Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads

(Optional) For preparation of many samples, the samples can be processed in a 96-well plate. Use a heat block compatible with 96-well plates or a thermal cycler for the 97±2°C incubation.

1. Prefill the appropriate number of 1.5-mL centrifuge tubes or wells of a 96-well PCR plate with 10 µL of Lysis Buffer, FS for all samples being tested, including any needed negative controls.
2. Transfer 90 µL of the resuspended Pathatrix™ bead pellet to each prefilled tube or well.
3. *(Optional)* For 96-well plates, seal the plate with non-optical film
4. Incubate sample(s) at 97±2°C for 12±2 minutes in a heating block or thermal cycler.
5. Remove the sample(s) from the heating block or thermal cycler and allow the sample(s) to cool to room temperature (23±5°C) for about 1 minute.
6. Proceed in one of the following ways:
   - Proceed directly to PCR with the MicroSEQ™ kit, if storage of the DNA samples before PCR is not required.
   - Process further for long-term DNA sample storage as follows:
     a. Place the DNA samples in a magnetic particle concentrator (if using microcentrifuge tubes) or magnetic plate (if using a 96-well plate).
     b. Leave the sample in the concentrator for 2±1 minutes.
     c. Remove up to 60 µL from the top of the sample and add it to a clean microcentrifuge tube or well of a 96-well plate.

        Avoid the magnetic particle pellet and any lysis debris while removing the DNA sample.
     d. Store the DNA sample below –18°C for up to 1 year.

        IMPORTANT! Do not freeze the DNA samples with Pathatrix™ beads.

The remaining 30 µL of the bead resuspension may be retained for further testing by direct plating on selective agar plates, if desired.

1. If the sample is not going to be plated immediately, transfer the full 30 µL into a clean 1.5-mL microcentrifuge tube, and store at 5±2°C for no more than 24 hours. Be sure to store the tube(s) away from magnets.
2. Streak approximately 10 µL onto each selective plate using a clean inoculating loop. If the sample was stored at 5±2°C, be sure that the sample has been resuspended before removing the 10-µL aliquots.
3. Incubate the plates at 37±1°C for 18–24 hours. See “Perform plating and biochemical assays to interpret positive results” on page 30 and the plates' manufacturers’ guides for further instructions and how to interpret the results.

5 Perform PCR

Overview

In this procedure, PCR-ready DNA is prepared from the captured Pathatrix™ beads, then PCR is performed using the prepared DNA, the MicroSEQ™ Salmonella spp. Detection Kit and RapidFinder™ Express Software. Individual samples from PCR-positive pooled samples are retested, and confirmation testing is performed on individual samples.

Refer to “Materials for PCR detection” on page 10 for kit contents and other required materials.

MicroSEQ™ assay beads contain all the components necessary for the real-time PCR: probe and primers for the target of interest, enzyme, and other buffer components. The assay beads also contain an internal positive control (IPC) probe, primers, and template to monitor for PCR inhibition.

A Pathogen Detection Negative Control is included in the kit. Unknown samples and positive control samples are provided by the investigator.

Workflow

Create or edit a run file in RapidFinder™ Express Software ▼	
Prepare the assay beads ▼ **Set up the PCR reactions** ▼	
Load and balance the instrument, then run the reactions ▼	
View results and data analysis	

Important procedural guidelines for PCR

Software

RapidFinder™ Express Software determines the Run Layout (plate layout) during creation of the run file, therefore it must be set up before distributing DNA samples to the assay beads.

For additional information, refer to the *Applied Biosystems™ RapidFinder™ Express Software Quick Reference* (Pub. No. 4480999) or the online help within the software.

Sample handling

- Use a new pipette tip for each sample.
- If you mix the assay beads with the DNA samples by pipetting up and down, keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
- Follow the recommendations in "Good laboratory practices for PCR" on page 43.

MicroAmp™ tube strips

- Follow these instructions to ensure proper storage of the tube strips:
  - Cut the storage pouch at the notch above the resealable strip.
  - Always reseal the storage pouch with desiccant, and replace at 5±3°C.
- 8-tube strips can be cut apart with scissors.

  If necessary, trim any remaining connector material from the cut to allow a better fit against adjacent tubes in the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips.
- MicroAmp™ Tube Strips are labeled 1–8 on the side of the tubes, to orient tube strips during handling.

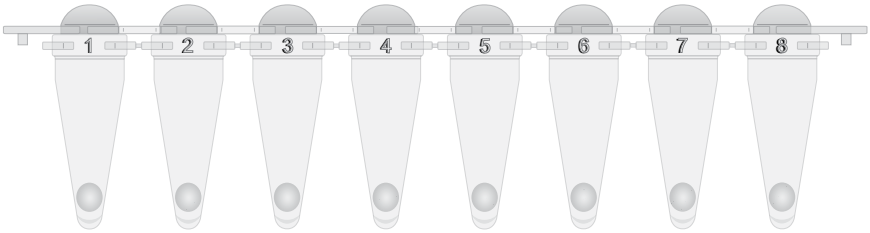


Figure 5 MicroAmp™ Tube Strip labeling

The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

If necessary for visual reference from above, mark the tab at one end of the cap strip. Do not mark any of the caps (this could interfere with real-time PCR detection).

- Seal the tubes with the transparent, optical cap strips provided in the kit. Do not use colored caps or tubes for real-time PCR reactions, because they may affect dye-signal readings during real-time PCR.
- Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.

- Use the MicroAmp™ 96-Well Base and the MicroAmp™ Cap Installing Tool to seal the assay tubes with the optical cap strips. This avoids collapsing, bending, or misaligning the tubes.

  Confirm that the strips are straight and that each tube is in line with the adjacent tube.
- Use a plate adapter for vortexing the tube strips, or hold the strips in the MicroAmp™ 96-Well Base while vortexing.

Create or edit a run file in RapidFinder™ Express Software

The RapidFinder™ Express Software is designed for use on the Applied Biosystems™ 7500 Fast Real-Time PCR System and must be set up before aliquoting samples.

On the main page of the RapidFinder™ Express Software, select **Create/Edit a Run File**  , and enter the target pathogen, number of samples, replicates, and positive and negative controls for each target at the prompts.

The software determines the sample layout based on the information entered and creates a run file.

Prepare the assay beads

Follow the plate layout determined by the RapidFinder™ Express Software.

1. Transfer the appropriate number of individual tubes or 8-tube strips from the storage pouch to a 96-well base at room temperature (23±5°C).
2. If required by the plate layout, place empty MicroAmp™ Fast 8-Tube Strips (or partial strips) to balance the tray when the assay tubes are placed in the instrument later.

Set up the PCR reactions

For step-by-step instructions, select **Pipette Samples** on the main page in RapidFinder™ Express Software.

1. Following the layout determined by RapidFinder™ Express Software, distribute the DNA sample lysates to MicroSEQ™ assay beads as described in the following table.

DNA sample type	Procedure
Frozen DNA samples (with Pathatrix™ **beads removed** and DNA frozen as described in Chapter 4, "Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads")	1. Just before use, completely thaw the DNA sample on ice. 2. Before opening, centrifuge briefly to remove condensation from the tubes or plates and avoid cross contamination. 3. Add DNA sample to the MicroSEQ™ assay bead: • **For samples from chocolate or cocoa matrices:** combine 5 µL of the sample with 25 µL of nuclease-free water, mix, then add to an assay bead. • **For all other matrices:** transfer 30 µL of the sample to an assay bead.
DNA samples that have not been frozen (with Pathatrix™ **beads still present**)	1. Place the DNA samples in a magnetic particle concentrator (if using microcentrifuge tubes) or magnetic plate (if using a 96-well plate). 2. Leave the sample in the concentrator for 2±1 minutes. 3. Add DNA sample to the MicroSEQ™ assay bead avoiding the magnetic particle pellet and any lysis debris at the bottom of the tube. • **For samples from chocolate or cocoa matrices:** carefully remove 5 µL from the top of the sample and combine with 25 µL of nuclease-free water, mix, then add to an assay bead. • **For all other matrices:** carefully transfer 30 µL from the top of the sample to an assay bead. 4. *(Optional)* Transfer 30 µL of the remaining sample to a clean microcentrifuge tube, avoiding the magnetic particle pellet and any lysis debris at the bottom of the tube, and store the DNA sample below –18°C for potential reanalysis.

2. Following the layout determined by RapidFinder™ Express Software, add 30 µL of the Pathogen Detection Negative Control and any positive control samples to the appropriate MicroSEQ™ assay beads at room temperature (23±5°C), and mix by gently pipetting up and down a few times.

   Assay beads dissolve in 1–5 seconds.

   Alternatively, vortex the assay tubes after they are capped, as described in step 5.

3. Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit.

4. Mark or label one end of the strip cap (but not directly over any one cap) to maintain the strip orientation when transferring the tubes to the instrument tray.

5. Make sure that the reactions are thoroughly mixed: if reactions were not previously mixed during the pipetting step, vortex to mix.

6. Make sure that the reagents are at the bottom of tubes: briefly centrifuge the strip tubes at 200–600 × *g* for about 20 seconds using a centrifuge with a plate adapter or a benchtop microcentrifuge with an 8-strip PCR tube adapter.

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. Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout.

   Use the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips in the instrument.

   Be sure to load empty low profile PCR tubes as directed by the software (Figure 6).

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

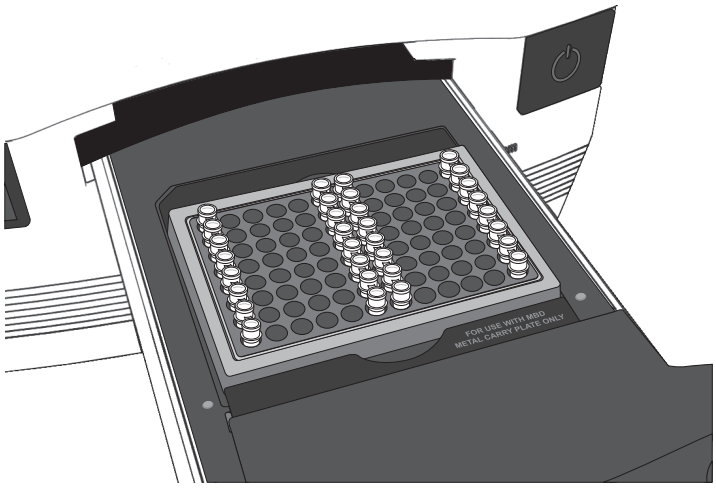


Figure 6 7500 Fast instrument tube layout

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.

Next steps after viewing results

Proceed as indicated in the following table, according to the RapidFinder™ Express result.

Result icon[1]	Result	Action
	Positive result	Pooled samples: proceed to "Retest individual samples after a positive pooled sample" on page 30. Individual samples: proceed to "Perform plating and biochemical assays to interpret positive results" on page 30.
	Negative result	Proceed to "Test result interpretation and classification" on page 31.
	Result warning	Proceed to "Retest after a PCR Result Warning" on page 31.

[1] RapidFinder™ Express displays results pictorially.

6 Confirm positive results

Retest individual samples after a positive pooled sample

Some applications require retesting of individual samples to determine which sample was the cause of the positive pooled sample. Retest individual samples as follows.

1. Rewarm individual samples that were stored as described in "Transfer the enriched culture to the Sample Vessel" on page 16 to 34–38°C, then transfer 10 mL to a Sample Vessel.

2. Proceed immediately to "Load and run the sample" on page 17 and follow the procedure through "Perform plating and biochemical assays to interpret positive results" on page 30.

Perform plating and biochemical assays to interpret positive results

Samples identified as PCR-positive may be confirmed by plating retained unlysed Pathatrix™ beads from Chapter 4, "Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads" onto selective agar plates, followed by biochemical and serological methods (ISO 6579:2017 or FDA BAM Chapter 5).

Note: If individual samples are used for confirmation, confirmation using pooled samples is optional.

If PCR is not used to identify *Salmonella*-positive samples, then the beads from pooled samples or individual samples can be plated directly onto selective agar plates [Pub. No. MAN0006975 (10-pooled sample) or MAN0006974 (individual samples)] and confirmed by biochemical and serological methods (ISO 6579:2017 or FDA BAM Chapter 5).

1. Streak 10 µL (one loopful) of the unlysed Pathatrix™ bead suspension from Chapter 4, "Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads" onto a well-dried XLD agar plate, and streak an additional 10 µL onto a second selective agar plate of choice.

   IMPORTANT! We recommend streaking, instead of spreading, to generate isolated colonies.

   The second selective agar plate can be any other solid selective medium complementary to XLD as long as it is appropriate for the isolation of lactose-positive *Salmonella*, *Salmonella typhi*, and *Salmonella paratyphi* strains. See Table 7 on page 11 for examples.

   Incubate the second selective agar plate according to the manufacturer's instructions.

2. Allow the plates to dry for 10 minutes, then invert and incubate at 34–38°C for 18–24 hours.

A presumptive positive is identified as any typical or atypical growth on the selected agar plates that would indicate *Salmonella* spp. colonies. Any presumptive positive isolate should be subsequently confirmed by sub-culture, followed by the appropriate biochemical and serological tests detailed in ISO 6579:2017.

Test result interpretation and classification

Once confirmed, the results are reported as:

- *Salmonella* spp. **Detected** in 25–325 g (sample matrices)
- *Salmonella* spp. **Not detected** in 25–325 g (sample matrices)

Retest after a PCR Result Warning

Result Warnings are shown when the internal positive control (IPC) in a reaction fails to amplify, and the target sequence also fails to amplify. Result Warnings are usually the result of PCR inhibition due to carry over of inhibitors from the sample preparation steps of the workflow.

If a Result Warning is obtained on a sample, re-test that sample at a 1:6 dilution using the following procedure.

For more information about how to view and interpret amplifications plots (optional), see “Investigate warning results or failed runs in SDS Software” on page 34.

1. Prepare for real-time PCR as described in “Create or edit a run file in RapidFinder™ Express Software” on page 26 and “Prepare the assay beads” on page 26.

2. Retrieve the retained DNA sample that was stored as described in Chapter 4, “Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads”.

   Fully thaw the DNA sample on ice, if necessary.

3. Dispense 25 µL of microbiology grade water to a real-time PCR sample tube from the MicroSEQ™ Salmonella spp. Detection Kit, then immediately add 5 µL of the thawed DNA sample.

4. Dispense negative control(s) and positive control(s) following the layout determined by RapidFinder™ Express Software.

   Note: Dilution of the controls is not required.

5. Mix samples or controls with the lyophilized MicroSEQ™ assay beads as described in “Set up the PCR reactions” on page 27, and continue the real-time PCR detection procedure.

A

Troubleshooting

General troubleshooting

Observation	Possible cause	Recommended action
Enriched culture is curdled.	Samples may be slightly acidic or alkaline (for example, milk powders, fermented milk samples, etc.).	Correct the pH of the sample-media mixture before enrichment. 1. Mix the food sample with appropriate enrichment media. 2. Incubate for 60±5 minutes at room temperature (23±5°C). 3. Homogenize the sample and determine the pH. 4. Adjust the pH to 6.8±0.2, and mix well before determining the final pH.
		Use Pathatrix™ Foam Filters (Cat. No. PFF) and the Pathatrix™ 5 Pooling Kit (Cat. No. POOL510MLN) or Pathatrix™ 10 Pooling Kit (Cat. No. POOL1010MLN) when sampling curdled enriched cultures.
PBS removed from the captured Pathatrix™ beads is not clear	Debris from the food sample has been carried over into the elution tube.	Perform a manual PBS wash on the Pathatrix™ beads as described in "Capture the Pathatrix™ beads" on page 20.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipet the positive control into all positive control wells.
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.

Observation	Possible cause	Recommended action
In negative control wells, no IPC signal is detected, but a target-specific signal is detected	Carryover contamination caused target signal in negative control wells.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
	Additionally, no IPC signal in negative control wells can be caused by: • A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification.	To determine whether IPC amplification is a problem, examine unknown wells for an IPC signal. If an IPC signal is present, IPC amplification is not a problem.
In unknown wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	Dilute 5 µL of the sample with 25 µL of nuclease-free water to dilute PCR inhibitors, and repeat the assay as described in "Retest after a PCR Result Warning" on page 31. If PCR remains inhibited, repeat the sample preparation.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
Multicomponent plot signals for FAM™, VIC™, and ROX™ detectors increase/decrease during cycles 1–15, but the amplification curve and result are not affected (this observation applies to **View in SDS** mode).	Incomplete mixing and dissolution of the lyophilized bead with sample or control.	After addition of 30 µL of sample or Pathogen Negative Control to the bead and capping the tubes: 1. Vortex strips at high speed for about 10 seconds, and centrifuge the strips at 200–600 × *g* for about 10 seconds. 2. Vortex the strips again on high speed for about 10 seconds, and centrifuge the strips at 200–600 × *g* for about 1 minute. Ensure that all liquid is at the bottom of the tubes and the beads are fully dissolved before proceeding.
Replicate results for a sample are inconsistent.	All replicate wells for a sample do not have the same result.	If more than two replicates yield the same result (for example, 2 of 3 replicates are negative, but 1 replicate is positive), refer to your laboratory protocol to determine whether to repeat the assay using fresh samples and reagents. If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.

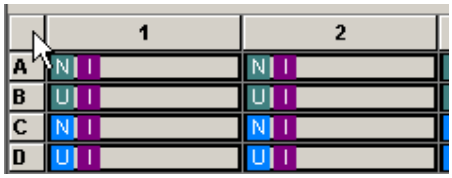
Observation	Possible cause	Recommended action
Amplicon contamination.	• Contamination was introduced into the PCR clean area from post-amplification reaction tubes that were either opened in the clean area or brought into the PCR clean area from contaminated gloves or solutions. • Contamination was introduced into the real-time PCR instrument from crushed and broken PCR reaction tubes.	To confirm amplicon contamination, perform the following experiment: Prepare negative control samples using at least one 8-tube strip of MicroSEQ™ Assay Beads. 1. Divide the assay beads into two sets. a. To the first set of assay beads, add 30 µL of nuclease-free water. b. To the second set of assay beads, add 29 µL of nuclease-free water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. No. 18054-015). 2. Run samples on the 7500 Fast Real-Time PCR Instrument using SDS software and select **Fast 7500** run mode. 3. Under the instrument tab: • Select **Add Step** to stage 1 of the PCR cycle that consists of 10 minutes at 50°C. • Extend the 95°C step from 20 seconds to 10 minutes. Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples. If the instrument block was contaminated, consult the *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve* (Pub. No. 4347825) and/or contact a service representative to clean the instrument.

Investigate warning results or failed runs in SDS Software

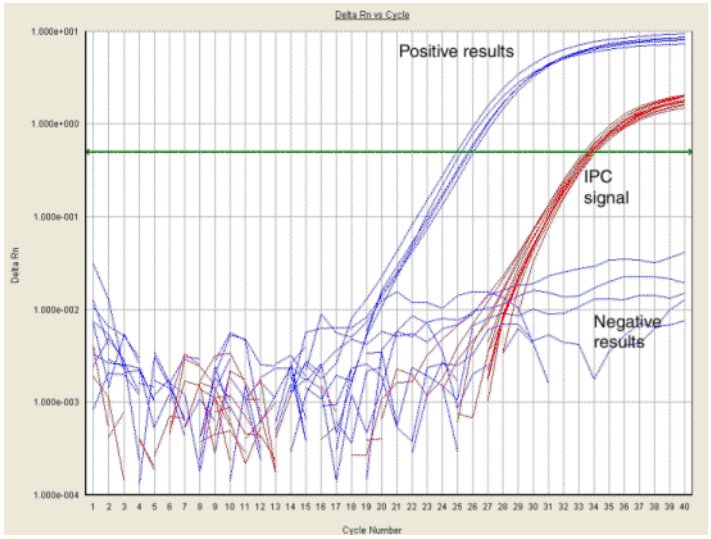
IMPORTANT! If you modify a RapidFinder™ Express Software run file in the SDS Software, you cannot open the run file again in the RapidFinder™ Express Software. To avoid altering a RapidFinder™ Express Software run file, save the run file under a new name in the SDS Software before performing any actions, as described below.

1. Open the run file in the SDS Software by one of the following methods:
   - From **View Results** in the RapidFinder™ Express Software, select and open the run file, then click **View in SDS**.
   - Open the run file in the SDS Software.

2. Select **File ▸ Save As**, then save the run file under a new name.
3. Select the **Results** tab.
4. Select the **Amplification Plot** tab.
5. Select all locations by clicking the top left corner of the layout.



6. Examine the Amplification Plot in Data mode of Delta Rn vs Cycle (displayed by default).



- To examine the signal for only tubes of interest, **Ctrl + Click** the locations below the plot.
- To examine the signal for only the IPC or a specific target, select the signal of interest from the Detector list at the top right of the plot.

- Refer to "Interpretation of the amplification plot for samples with a Result Warning" on page 36.

7. When you finish viewing the run file, exit the SDS software:
   - If you accessed the run file from the RapidFinder™ Express Software, in the SDS Software, select **File ▸ Return to RapidFinder™ Express Software**.
   - If you opened the run file directly in the SDS software, in the SDS Software, select **File ▸ Exit**.

Interpretation of the amplification plot for samples with a Result Warning

For the IPC and the pathogen target detector, observe if the curve displayed in the Amplification Plot crosses the highlighted horizontal line, sometimes referred to as the "threshold" line.

IMPORTANT! The RapidFinder™ Express Software will automatically select the appropriate threshold values for each detector. Unless advised by a Thermo Fisher Scientific representative, do not change these values.

Additionally, the "highlighted horizontal line," as described above, will appear only when one of the detectors is selected. If "All" detectors are selected, the horizontal line will not be in the correct location for proper visual identification of the sample(s).

Observation	Possible cause	Recommended action
IPC curve **does not** cross the threshold line.	IPC in this sample was inhibited.	Add 5 µL the stored DNA sample lysate to 25 µL of nuclease-free water and repeat the PCR.
IPC and Target curves **do not** cross the threshold line.	PCR inhibition in the sample.	Add 5 µL the stored DNA sample lysate to 25 µL of nuclease-free water and repeat the PCR.
IPC curve **does** cross the threshold line and target curve **does not** cross the threshold line.	No PCR inhibition. Sample is negative for target detection.	None needed; result is negative.

B Alternative enrichment methods

See Appendix C, "AOAC *Performance Tested Methods*℠ Certification" for details about matrices used in the AOAC validation study.

Prepare potentially acidic/alkaline samples for enrichment

Samples which potentially deviate from neutral pH (for example, milk powders, fermented milk samples, etc.) should be prepared as follows.

1. Prepare the sample according to the sample enrichment protocol.
2. Incubate for 60±5 minutes at room temperature.
3. Homogenize the sample and determine the pH.
4. If necessary, adjust the pH to 6.8±0.2, and mix well before determining the final pH.

Enrich dairy samples

Dairy samples include milk-derived products such as:

- Fermented milk products (cheeses, yogurt)
- Dry milk products (infant milk powder, non-fat powdered milk)
- Liquid milk products (pasteurized milk)
- Raw milk products (unpasteurized cheeses, liquid raw milk)

Dairy and milk powder samples can benefit from the following alternative enrichment strategy.

- For dairy samples, use prewarmed Buffered Peptone Water (BPW) supplemented with Brilliant Green (0.002%) as the enrichment media. Additionally, see "Prepare potentially acidic/alkaline samples for enrichment" on page 37.
- Incubate dairy samples at 34–38°C for 20±2 hours.
- Pre-warm the BPW supplemented with Brilliant Green to 34–38°C before use.

Enrich chocolate and cocoa-based samples

All chocolate or cocoa-based samples can benefit from an alternative enrichment.

- Use pre-warmed sterile UHT skim milk or pre-warmed non-fat dry milk reconstituted in Molecular Biology Grade water or pre-warmed Buffered Peptone Water as the enrichment media. Ensure the addition of Brilliant Green (0.018 g/L) to UHT skimmed milk or NFDM for products with high-background microflora.
- Incubate chocolate and cocoa-based samples, as described in the protocol, at 37°C for 20-28 hours when enriched in UHT skimmed milk or NFDM or for 22-30 hours when enriched in BPW.

C AOAC *Performance Tested Methods*℠ Certification

Table 8 *Performance Tested Methods*℠ Certification of the workflow

Certification	
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 090203C	Pathatrix™ Salmonella spp. Pooling Kits For more information, please refer to certificate **090203C**.
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001	MicroSEQ™ Salmonella spp. Detection Kit For more information, please refer to certificate **031001**.

The detection of *Salmonella* spp. using Pathatrix™ Salmonella spp. Pooling Kits and the MicroSEQ™ Salmonella spp. Detection Kit has earned the AOAC *Performance Tested Methods*℠ Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment in Prewarmed Buffered Peptone Water
- Pathatrix™ Salmonella spp. Pooling Kits
- MicroSEQ™ Salmonella spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 9 for characteristics) with RapidFinder™ Express Software v2.0 or later.

Table 9 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

Table 9 7500 Fast Real-Time PCR Instrument characteristics *(continued)*

Characteristics	7500 Fast Real-Time PCR Instrument
Thermal uniformity	±1°C
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples as described in "Perform plating and biochemical assays to interpret positive results" on page 30.

Reference method	Matrix
USDA/FSIS MLG 4.10	Cooked ham up to 325 g
US FDA BAM, Chapter 5	• Fresh diced tomatoes, 25 g • Chocolate, 25 g

D

Supplemental information

Description of target microorganism

More than 2,400 *Salmonella* serotypes have been reported, all of which are potentially pathogenic. *Salmonella* is a frequently reported cause of food-borne illness, occurring in both epidemics and in isolated cases. *Salmonella* bacteria are the causative agent for Salmonellosis. Outbreaks have been associated with raw meats and poultry, eggs, milk and dairy products, seafood, coconut sauces, salad dressings, cocoa, chocolate, spices, frozen products, and vegetables such as hot peppers.

Audience

The Pathatrix™ Salmonella spp. Pooling Kits and the MicroSEQ™ Salmonella spp. Detection Kit are for professional use only and are intended for use by qualified users interested in determining the presence/absence of *Salmonella* spp. in food and environmental samples. Users may include, but are not limited to, food producers, food processors, food manufacturers, retailers, and microbiology testing laboratories.

Applicability

The Pathatrix™ Salmonella spp. Pooling Kits has earned the *Performance Tested Methods*℠ (PTM) Certification from the AOAC Research Institute (AOAC-RI) for use with food matrices including cooked ham, chocolate, and chopped tomatoes. The test operates in conjunction with a pooled Pathatrix™ workflow. A standard 25-g food sample is hand mixed briefly with 225 mL of enrichment medium or a 325 g of food sample is hand mixed briefly with 2925 mL enrichment medium. The sample is then incubated at 34–38°C for 20±2 hours, or 20-28 hours for cocoa and chocolate products enriched in UHT skimmed milk or NFDM, or 22-30 hours for cocoa and chocolate products enriched in BPW. After incubation, a 5-mL sample aliquot is combined with up to 9 additional 5-mL sample aliquots and the resulting 50-mL pooled sample is then subjected to a 15-minute Pathatrix™ Auto Instrument capture cycle. The kit is AOAC-RI approved for use with agar-based detection.

The MicroSEQ™ Salmonella spp. Detection Kit has earned the PTM Certification from the AOAC-RI for use with 11 different food matrices consisting of raw ground beef, raw chicken, raw shrimp, Brie Cheese, shell eggs, cantaloupe, chocolate, black pepper, dry infant formula, dry pet food, and peanut butter; and with 5 environmental surfaces consisting of sealed concrete, stainless steel, plastic, ceramic tile, and rubber.

The method modification linking the Pathatrix™ Salmonella spp. Pooling Kits to the MicroSEQ™ Salmonella spp. Detection Kit has been approved by the AOAC-RI for use with fresh diced tomatoes, chocolate, and deli ham.

Sampling protocol

The standard food sample size used in the Pathatrix™ system is 25 g of food diluted with 225 mL of enrichment medium or 325 g of chocolate diluted with 2925 mL of enrichment medium. We recommend that sub-samples for pooling are processed immediately or, if storage is required, that the samples are refrigerated (5±3°C). Samples should be rewarmed to 34–38°C prior to analysis with the Pathatrix™ Auto system. The remaining enriched sample should be stored at 5±3°C up to 32 hours until the results of the pooled sample have been determined.

Kit sensitivity

The Pathatrix™ Salmonella spp. Pooling Kits is a sample preparation method for presence/absence testing based on the detection of as few as 1–10 cfu (colony forming units)/25–325 g of food sample. The limitation of the Pathatrix™ Salmonella spp. Pooling Kits is in the ability of the target to reproduce in the enrichment medium, be captured by the magnet, and subsequently be detected by real-time PCR or selective agar.

CAUTION! The Pathatrix™ Salmonella spp. Pooling Kits used in conjunction with the MicroSEQ™ Salmonella spp. Detection Kit has been evaluated on fresh diced tomatoes, chocolate, and deli ham. Given the wide variety of products and manufacturing procedures, we recommend that you check that the composition of the matrices to be tested does not affect the reliability of the results.

A negative result does not guarantee the absence of target organism in the original sample and may be due to the inability of the organism to adequately reproduce to required levels in the enrichment medium (with subsequent outgrowth on selective agar plates) potentially due to, but not limited to, competitive microflora, sub-lethal injury, or matrix inhibition.

Operating conditions

Table 10 Pathatrix™ Auto Instrument and 7500 Fast Real-Time PCR System operating conditions (for indoor use only)

Condition	Acceptable range
Temperature	10–40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
Altitude	Not exceeding 2,000 m (6,500 ft) above sea level

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**.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps for PCR tubes come in strips of 8 or 12.

Safety

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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.

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*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

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.

References

Food Safety and Inspection Service (USDA). Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products. MLG 4.05. Microbiology Laboratory Guidebook.

EN ISO 6579-1:2017. Microbiology of the food chain -– Horizontal method for the detection, enumeration and serotyping of *Salmonella* -- Part 1:Detection of *Salmonella* spp.

Narang, N. and Cray, W.C. 2006. Evaluation of Hand Mixing of Ground Beef and Poultry Samples as an Alternative to Stomaching for the Detection of *Salmonella*. *Food Protection Trends*. 26:14–19.

USFDA Bacteriological Analytical manual (BAM) Chapter 5: *Salmonella*. February 2020. **http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm**