Pathatrix™ *Salmonella* spp. Kits Linked to MicroSEQ™ PCR or Selective Agar Detection

NF VALIDATION™-certified workflows for pooled or individual food samples

for use with:
Pathatrix™ 10-Pooling Salmonella spp. Kit
Pathatrix™ Salmonella spp. Kit
Pathatrix™ Auto Instrument
MicroSEQ™ Salmonella spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR System
RapidFinder™ Express Software

**Catalog Numbers** APS500P, APS50, 4403930
**Publication Number** MAN0009585
**Revision** E.0

For testing of Food and Environmental samples only.
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Revision history: Pub. No. MAN0009585 [English]

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>E.0</td>
<td>01 March 2019</td>
<td>• Added Microbact GNB 24E Kit, which is required for confirmation testing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Updated references.</td>
</tr>
<tr>
<td>D.0</td>
<td>07 Sept 2017</td>
<td>• Corrected the enrichment time for chocolate or cocoa-based samples to 18–22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Minor clarifications.</td>
</tr>
<tr>
<td>C.0</td>
<td>24 October 2016</td>
<td>• Added instructions for new validated matrices: cocoa and chocolates.</td>
</tr>
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<td></td>
<td></td>
<td>• Reorganized guidelines for PCR to align with current style and template.</td>
</tr>
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<td></td>
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<td>• Corporate and legal updates.</td>
</tr>
<tr>
<td>B.0</td>
<td>September 2014</td>
<td>• In “Capture the Pathatrix™ beads” on page 19, corrected the wash procedure; now includes 1X-PBS wash.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Updated corporate trademark and copyright statements.</td>
</tr>
<tr>
<td>A.0</td>
<td>February 2014</td>
<td>New document.</td>
</tr>
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</table>

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

CAUTION! *Salmonella* spp. is a Biosafety Level 2 (BSL-2) organism [excluding *S. Typhi* and *S. Paratyphi*, which are both Biosafety Level 3 (BSL-3)]. Care must be taken when handling samples that may contain salmonellae. Laboratory personnel must be adequately trained to handle pathogens before being permitted to analyze samples for *Salmonella* spp. Laboratory personnel must wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. Waste should be disposed of in compliance with local and national legislation as appropriate.

This guide describes the following NF VALIDATION™-certified workflows for detection of *Salmonella* spp. in food samples:

1. Enrichment of food samples.
2. Immunomagnetic separation (IMS) and capture of *Salmonella* spp. using the Pathatrix™ Auto Instrument and one of the following kits:
   - The Pathatrix™ 10-Pooling Salmonella spp. Kit (Cat. No. APS500P), for pooled enriched cultures (up to 10 cultures).
   - The Pathatrix™ Salmonella spp. Kit (Cat. No. APS50), for individual enriched cultures.
3. Detection of *Salmonella* spp. using one of the following methods:
   - Direct plating of captured Pathatrix™ bead-bacteria complexes on selective agar plates.
4. Retesting of individual samples following a positive pooled sample result, using the same detection method as for the pooled samples.
5. Confirmation testing of individual positive samples as described for each detection method.

Ensure that all instruments and equipment are properly installed and calibrated. For calibration information, see the documentation that is provided with your instruments.

See Appendix B, “NF VALIDATION™ by AFNOR Certification” for detailed information about the validated matrices and food categories.

Go to thermofisher.com/foodsafety for a list of certified workflows for detection of *Salmonella* spp.
Procedure overview

The procedure is illustrated in Figure 1. A 25-g food sample is homogenized with 225 mL of enrichment media and incubated for 18–22 hours at 37°C. For pooled samples, a 5-mL aliquot of enriched culture is combined with up to 9 additional 5-mL aliquots of enriched culture of the same matrix type, for a maximum pooled sample volume of 50 mL (see Table 9 in Appendix B, “NF VALIDATION™ by AFNOR Certification”).

Pooled enriched cultures are then subjected to a ~15-minute Pathatrix™ Auto Instrument cycle with anti-Salmonella spp. antibody-coated Pathatrix™ beads, to separate Salmonella spp. from the sample matrix and background flora. The Salmonella-Pathatrix™ bead complexes are magnetically captured and resuspended in 120 µL of Nuclease-free Water.

- For PCR detection, 90 µL of the resuspended beads are mixed with 10 µL of Lysis Buffer and subjected to heat lysis at 97°C for 10 minutes. Pathatrix™ beads are removed from the lysate using a magnetic particle concentrator or magnetic plate. The resulting lysate undergoes PCR using the MicroSEQ™ Salmonella spp. Detection Kit.
- For selective agar detection, 10 µL of the (unlysed) resuspended Pathatrix™ bead-bacteria complexes are streaked onto XLD and Oxoid Brilliance™ Salmonella Agar.

Positive pooled samples are retested individually by reprocessing 10-mL samples of stored individual enriched cultures on the Pathatrix™ Auto Instrument with the APS50 kit, and continuing through the chosen detection procedure. Independent confirmation testing is performed only on positive individual samples, according to the detection method chosen.
Samples can be individually tested without pooling using the APS50 kit starting from 10 mL of individual enriched culture, following the same detection and confirmation procedures as for pooled samples, according to the detection method chosen.

---

**Pool sample testing**

- Individual samples (up to 10)
- Individual sample enrichment
- Enriched cultures
- Pooling (10 × 5-mL samples)
  - 50-mL pooled sample (1 × Pathatrix™ Test)

**Individual sample testing**

- Individual sample
- Enriched culture
- 10-mL individual sample
  - 1 × Pathatrix™ Test

NOTE: retesting of individual samples from a positive pooled sample must use the same detection method as for the pooled sample.

Figure 1 Procedure overview
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 enrichment of food samples

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator, 37±1°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Homogenizer Laboratory Blender, one of the following, or equivalent</td>
<td>DB5000A, DB4100A, DB4150A</td>
</tr>
<tr>
<td>Homogenizer bags appropriate for the sample type and size</td>
<td>For DB4100A or DB4150A: DB4011A, DB4012A, DB4013A, DB4014A</td>
</tr>
</tbody>
</table>

**Enrichment media appropriate for the sample type:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered peptone water (BPW)</td>
<td>DF1049, CM1211B, or equivalent</td>
</tr>
<tr>
<td>Brilliant green (CAS 633-03-4)</td>
<td>Fisher Scientific™ B422-25, or equivalent</td>
</tr>
<tr>
<td>Ultra-high temperature (UHT) processed skim milk</td>
<td>Food retail store</td>
</tr>
</tbody>
</table>

### Materials for immunomagnetic separation

**Table 1** Pathatrix™ 10-Pooling Salmonella spp. Kit (Cat. No. APS500P; 50 cartridge runs; 50 pools of 10 samples; 500 samples)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pre-sterilized Sample and Elution Vessel Packs</td>
<td>50 each[^1]</td>
<td>Room temperature</td>
</tr>
<tr>
<td>• Pre-sterilized Capture Phase Packs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pre-sterilized Flat Cap Lids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^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  Pathatrix™ Salmonella spp. Kit (Cat. No. APS50; 50 cartridge runs; 50 samples)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pre-sterilized Sample and Elution Vessel Packs</td>
<td>50 each(^{[1]})</td>
<td>Room temperature</td>
</tr>
<tr>
<td>• Pre-sterilized Capture Phase Packs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pre-sterilized Flat Cap Lids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Salmonella spp. Antibody-Coated Paramagnetic Beads(^{[2]})</td>
<td>2.5 mL</td>
<td>5±3°C(^{[3]})</td>
</tr>
</tbody>
</table>

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

Table 3  Required materials not included in the Pathatrix™ kits

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathatrix™ Auto Instrument, including Sample Vessel Holder and Elution Vessel Holder</td>
<td>PTXAUTOINS</td>
</tr>
<tr>
<td>/Optional/ Pathatrix™ Cartridge Rack; holds 5 Cartridges</td>
<td>ACARTRACK</td>
</tr>
<tr>
<td>/Optional/ Pathatrix™ Elution Vessel Holder; holds 5 Elution Vessels(^{[1]})</td>
<td>ATUBERACK</td>
</tr>
<tr>
<td>PBS, 10X, pH 7.4 Dilute 1:10 in molecular biology-grade water (Cat. No. AM9932 or equivalent) prior to use</td>
<td>AM9624 or AM9625</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>AM9932</td>
</tr>
</tbody>
</table>

\(^{[1]}\) Additional Elution Vessel Holders may be purchased; however, an Elution Vessel Holder is included with the Pathatrix™ Auto Instrument.

Materials for PCR detection

Table 4  Materials for PCR-ready DNA sample preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating block, 97±2°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile 1.5-mL microcentrifuge tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>/Optional/ 96-well PCR plates and non-optical film (for processing large numbers of samples)</td>
<td>4346906 and 4306311, or equivalent</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>AM9938</td>
</tr>
<tr>
<td>Lysis Buffer, FS</td>
<td>4480724</td>
</tr>
<tr>
<td>Item</td>
<td>Source</td>
</tr>
<tr>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Magnetic-particle concentrator:</td>
<td></td>
</tr>
<tr>
<td>DynaMag™-2 Magnet (for use with microcentrifuge tubes)</td>
<td>123-21D</td>
</tr>
<tr>
<td>Magnetic rack (for use with 96-well plate; for example, Pathatrix™ Magnetic Plate or Magnetic Stand-96)</td>
<td>MAGNETICPLATE or AM10027</td>
</tr>
</tbody>
</table>

**Table 5** MicroSEQ™ Salmonella spp. Detection Kit [96 reactions; Cat. No. 4403930]

<table>
<thead>
<tr>
<th>Contents</th>
<th>Amount</th>
<th>Cap color</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp. Assay Beads, 8-tube strips</td>
<td>12 strips (96 tubes)</td>
<td>Green (rack)</td>
<td>5±3°C Protect from light and moisture.¹¹</td>
</tr>
<tr>
<td></td>
<td>1 rack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroAmp™ Optical 8-Cap Strips</td>
<td>12 strips (96 caps)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Pathogen Detection Negative Control [²]</td>
<td>1.5 mL</td>
<td>Red</td>
<td>5±3°C</td>
</tr>
</tbody>
</table>

¹¹ 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.

² The Pathogen Detection Negative Control is included in a separate box and may be shipped separately.

**Table 6** Required materials not included with the MicroSEQ™ kit

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instruments and equipment</td>
<td></td>
</tr>
<tr>
<td>Applied Biosystems™ 7500 Fast Real-Time PCR Instrument</td>
<td>A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative.</td>
</tr>
<tr>
<td>7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips</td>
<td>A29252</td>
</tr>
<tr>
<td>MicroAmp™ 96-Well Base</td>
<td>N8010531</td>
</tr>
<tr>
<td>MicroAmp™ Cap Installing Tool</td>
<td>4330015</td>
</tr>
<tr>
<td>MicroAmp™ Multi-removal Tool</td>
<td>4313950</td>
</tr>
<tr>
<td>Item</td>
<td>Source</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Benchtop microcentrifuge with 8-tube strip adapter</td>
<td>MLS</td>
</tr>
<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>Plate centrifuge</td>
<td></td>
</tr>
<tr>
<td>Laboratory mixer [Vortex mixer or equivalent]</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettors:</td>
<td>MLS</td>
</tr>
<tr>
<td>• Positive-displacement</td>
<td></td>
</tr>
<tr>
<td>• Air-displacement</td>
<td></td>
</tr>
<tr>
<td>• Multichannel</td>
<td></td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
</tr>
<tr>
<td>Aerosol-resistant pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>MicroAmp™ Fast 8-Tube Strip, 0.1-mL[1]</td>
<td>4358293</td>
</tr>
<tr>
<td>MicroAmp™ Optical 8-Cap Strip, 300 strips[1]</td>
<td>4323032</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td></td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>AM9938</td>
</tr>
</tbody>
</table>

[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.

Table 7  Materials for confirmation testing of PCR-positive samples

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator, 37±1°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile, disposable 10-µL loops</td>
<td>MLS</td>
</tr>
<tr>
<td>Xylose lysine deoxycholate (XLD) agar</td>
<td>MLS</td>
</tr>
<tr>
<td>Oxoid™ Brilliance™ Salmonella Agar</td>
<td>CM1092B</td>
</tr>
<tr>
<td>Oxoid™ Salmonella Selective Supplement</td>
<td>SR0194E</td>
</tr>
</tbody>
</table>

**Materials for additional confirmatory testing of characteristic colonies;** see "Confirmation testing of PCR-positive individual samples" on page 27.
Chapter 1 Overview
Required materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator, 37±1°C</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile, disposable 10-µL loops</td>
<td>MLS</td>
</tr>
<tr>
<td>Xylose lysine deoxycholate (XLD) agar</td>
<td>MLS</td>
</tr>
<tr>
<td>Oxoid™ Brilliance™ Salmonella Agar</td>
<td>CM1092B</td>
</tr>
<tr>
<td>Oxoid™ Salmonella Selective Supplement</td>
<td>SR0194E</td>
</tr>
</tbody>
</table>

Materials for confirmation after selective agar detection, according to the method chosen; see “Confirmation testing of positive individual samples” on page 29.
Enrich food samples

Refer to “Materials for enrichment of food samples” on page 8.

For preparation of initial suspensions, follow instructions of EN ISO 6579-1 and 6887-1 standards.

**IMPORTANT!** Use proper aseptic technique while handling samples to avoid cross-contamination.

1. Prewarm 225 mL of enrichment media per sample to 37±1°C, as indicated in the following table.

<table>
<thead>
<tr>
<th>Sample type[1]</th>
<th>Enrichment media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>BPW</td>
</tr>
<tr>
<td>Dairy samples, including milk-derived products</td>
<td>BPW + 0.002% Brilliant Green</td>
</tr>
<tr>
<td>Chocolate or cocoa-based samples</td>
<td>Sterile UHT skim milk + 0.002% Brilliant Green</td>
</tr>
</tbody>
</table>

[1] See Table 9 in Appendix B, “NF VALIDATION™ by AFNOR Certification”.

2. Add 225 mL of prewarmed media to 25 g (or 25 mL) of food sample in a homogenizer bag.

3. Process for 1–2 minutes in a homogenizer.
   *Hand massage foods that cannot be processed in a homogenizer (Narang et al, 2006).*

4. Incubate at 37±1°C under static conditions for the time indicated in the following table.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Enrichment time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat or dairy samples</td>
<td>Total of 18–22 hours</td>
</tr>
<tr>
<td>Chocolate or cocoa-based samples</td>
<td></td>
</tr>
</tbody>
</table>
IMS capture of *Salmonella* spp. with the Pathatrix™ Kits

The Pathatrix™ *Salmonella* spp. Kits, when used with the Pathatrix™ Auto Instrument, permit immunomagnetic separation (IMS) and capture and isolation of *Salmonella* spp. bacteria from pre-enriched food cultures.

- The Pathatrix™ 10-Pooling Salmonella spp. Kit format (Cat. No. APS500P) supports pooling of up to 10 enriched cultures into a single test (total pooled sample volume of 50 mL).
- The Pathatrix™ Salmonella spp. Kit (Cat. No. APS50) is also part of the NF-validated workflow. It can be used to reprocess 10-mL samples of individual enriched cultures after a positive pooled sample result. It can also be used for processing 10-mL samples of individual enriched cultures without pooling.

Instructions for processing samples with each kit are provided in this chapter. See “Materials for immunomagnetic separation” on page 8 for kit contents and other required materials.
**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 or selective agar detection**

**Important procedural guidelines**

- Use aseptic technique and good laboratory practices at all times.
- 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.

**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 (included with the Pathatrix™ Auto Instrument; see Figure 2).

2. Partially remove the lids from both vessels, making an opening large enough to allow the addition of enriched culture and wash buffer to the vessels.
3. Transfer the enriched culture to the Sample Vessel as indicated in the following table.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled sample</td>
<td>Prepare a pooled sample by transferring 5-mL aliquots from up to 10 individually enriched cultures to a Sample Vessel.</td>
</tr>
<tr>
<td><strong>Note:</strong> Pool enriched cultures only from the same matrix type[1].</td>
<td></td>
</tr>
<tr>
<td>Individual enriched culture for retesting after a positive pooled sample</td>
<td>Transfer 10 mL of stored individual enriched culture, rewarmed for 1–2 hours at 37±1°C, to a Sample Vessel.</td>
</tr>
<tr>
<td>Individual enriched culture (no pooling)</td>
<td>Transfer 10 mL of enriched culture to a Sample Vessel.</td>
</tr>
<tr>
<td>[Optional but recommended] Negative extraction control</td>
<td>Transfer 10 mL of sterile enrichment media to a Sample Vessel.</td>
</tr>
</tbody>
</table>

[1] Table 9 in Appendix B, “NF VALIDATION™ by AFNOR Certification”

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

**Note:** Do not store enriched cultures for more than 32 hours total.

**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 from the indicated kit into the spout on the lid of the Sample Vessel (Figure 2).

**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.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Use Pathatrix™ beads from this kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled sample</td>
<td>APS500P</td>
</tr>
<tr>
<td>Individual enriched culture for retesting after a positive pooled sample</td>
<td>APS50</td>
</tr>
<tr>
<td>Individual enriched culture (no pooling)</td>
<td>APS50</td>
</tr>
</tbody>
</table>
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.

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](image)

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. 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.

5. Place the Elution Vessel in the Sample Vessel Holder and allow it to remain there for approximately 1 minute.
6. While the Elution Vessel is still in the Sample Vessel Holder, carefully remove and discard the PBS without disturbing the captured Pathatrix™ beads.

7. 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. The Pathatrix™ bead suspension is ready for detection by real-time PCR (Chapter 4, “PCR detection and confirmation testing“) or selective agar plating (Chapter 5, “Selective agar detection and confirmation testing“).
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 9 for kit contents and other required materials.

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

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.

   **Note:** If you are retesting individual samples after a positive pooled result, concurrently streak 10 µL of the remaining resuspended Pathatrix™ beads onto selective agar plates for confirmation testing, as described in “Retest individual samples after a positive pooled sample” on page 26.

3. Incubate sample(s) at 97±2°C for 12±2 minutes in a heating block or thermal cycler.

4. 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.

5. 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.

**PCR with the MicroSEQ™ Salmonella spp. Detection Kit and RapidFinder™ Express Software**

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 Appendix C, “Good laboratory practices for PCR”.

**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](image)

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, in RapidFinder™ Express Software v1.1, select Pipette Samples on the main page.

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

<table>
<thead>
<tr>
<th>DNA sample type</th>
<th>Procedure</th>
</tr>
</thead>
</table>
| Frozen DNA samples (with Pathatrix™ beads removed and DNA frozen as described in “Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads” on page 21) | 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 x 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. 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 tube strips 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.

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.

<table>
<thead>
<tr>
<th>Result icon[1]</th>
<th>Result</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Positive]</td>
<td>Positive result</td>
<td>Pooled samples: proceed to “Retest individual samples after a positive pooled sample” on page 26. Individual samples: proceed to “Confirmation testing of PCR-positive individual samples” on page 27.</td>
</tr>
<tr>
<td>![Negative]</td>
<td>Negative result</td>
<td>No action required.</td>
</tr>
<tr>
<td>![Warning]</td>
<td>Result warning</td>
<td>Dilute 5 µL of the retained DNA sample with 25 µL of Nuclease-free Water and repeat the PCR.</td>
</tr>
</tbody>
</table>

[1] RapidFinder™ Express displays results pictorially.

Retest individual samples after a positive pooled sample

Pooled samples that test positive by PCR must be retested as individual samples, as follows:

1. Rewarm stored individual enriched cultures at 37±1°C for 1–2 hours.

2. Set up fresh Sample and Elution Vessels in a Sample Vessel Holder, and transfer 10 mL of the warmed individual enriched culture to individual Sample Vessels.

3. Process the individual cultures on the Pathatrix™ Auto Instrument starting at “Load and run the sample” on page 16 through “Capture the Pathatrix™ beads” on page 19.

4. Follow the procedure “Prepare a PCR-ready DNA lysate from captured Pathatrix™ beads” on page 21 with 90 µL of the Pathatrix™ bead suspension, through “PCR with the MicroSEQ™ Salmonella spp. Detection Kit and RapidFinder™ Express Software” on page 22.

5. Concurrently, streak the remaining unlysed Pathatrix™ bead suspension onto selective agar for confirmation testing.
   a. Streak 10 µL (one loopful) of the remaining unlysed Pathatrix™ bead suspension onto a well-dried XLD agar plate, and streak an additional 10 µL onto Oxoid™ Brilliance™ Salmonella Agar.

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

   b. Allow the plates to dry for 10 minutes, then invert and incubate at 37±1°C for 18–24 hours.
Confirmation testing of PCR-positive individual samples

Confirmation testing is performed only on individual samples, not pooled samples.

In the context of NF VALIDATION™ Certification, all individual samples identified as positive by the Pathatrix™ Auto Salmonella spp. Kit Linked to MicroSEQ™ Salmonella spp. Detection Kit method must be confirmed by one of the following tests:

- By using the conventional tests described in the methods standardized by CEN or ISO (including the purification step). The confirmation step must start from the enrichment broth (either BPW or UHT milk).
- By performing a confirmatory test on observed characteristic colonies from the plated unlysed Pathatrix™ beads from the individual retested samples (“Retest individual samples after a positive pooled sample” on page 26), as described in the following table.

<table>
<thead>
<tr>
<th>Characteristic colonies observed</th>
<th>XLD agar</th>
<th>Oxoid™ Brilliance™ Salmonella Agar</th>
<th>Confirmatory test</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>None required</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Microbact GNB 24E Kit (MB1131A)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
<td>Latex test (Oxoid™ DR1108)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Latex test (Oxoid™ DR1108) on colonies from Oxoid™ Brilliance™ Salmonella Agar</td>
<td></td>
</tr>
</tbody>
</table>

- Using any other method certified by NF VALIDATION™; the principle must be different from that of the Pathatrix™ Auto Salmonella spp. Kit Linked to MicroSEQ™ Salmonella spp. Detection Kit method (immunomagnetic separation and PCR). The detection protocol of the second validated method used for the confirmation shall be followed entirely. All steps which are before the step from which the confirmation is started shall be common to both methods (that is, enrichment in BPW at 37°C).

In the event of discordant results (positive with the alternative method, non-confirmed by one of the means described above, and in particular the latex test), the laboratory must follow the necessary steps to ensure the validity of the result obtained.

**Note:** Some strains of Salmonella belonging to the serovar Dublin may show a weak to nil magenta pigmentation, because of their low esterase activity.
Selective agar detection and confirmation testing

Refer to “Materials for selective agar detection” on page 12.

Important procedural guidelines

- Wear a facemask when weighing out powders.
- Take care when boiling agar prior to autoclaving, and wear heat-resistant gloves when handling hot flasks of liquid.

Detect *Salmonella* spp. by selective agar plating

**Note:** We recommend streaking the sample out to generate individual colonies, as opposed to spread plating.

1. Streak 10 µL of the unlysed Pathatrix™ bead suspension over a well-dried XLD agar plate and another 10 µL onto Oxoid™ Brilliance™ Salmonella Agar.

2. Allow the plates to dry for approximately 10 minutes, then invert and incubate at 37±1°C for 18–24 hours.

   A presumptive positive result is defined as the isolation of typical, suspicious, or atypical *Salmonella* spp. colonies on the agar plates used.

If a negative result is obtained from a pooled sample, the individual enriched cultures can be discarded, as further testing is not required.

If a positive result is obtained from a pooled sample, retest the individual enriched cultures to allow identification of which individual samples in the pool produced the positive result, as described in the next section.

**Note:** Some strains of *Salmonella* belonging to the serovar Dublin may show a weak to nil magenta pigmentation, because of their low esterase activity.

Retest individual samples after a positive pooled sample result

Pooled samples that test positive by selective agar must be retested as individual samples, as follows:
1. Rewarm stored individual enriched cultures at 37±1°C for 1–2 hours.

2. Set up fresh Sample and Elution Vessels in a Sample Vessel Holder, and transfer 10 mL of the warmed individual enriched sample to the Sample Vessel.

3. Process the individual samples on the Pathatrix™ Auto Instrument, starting at “Load and run the sample” on page 16 through “Capture the Pathatrix™ beads” on page 19.

4. Follow the procedure “Detect Salmonella spp. by selective agar plating” on page 28. A presumptive positive result is defined as the isolation of typical, suspicious, or atypical Salmonella spp. colonies on the agar plates used.

5. Proceed to “Confirmation testing of positive individual samples” in the next section.

**Confirmation testing of positive individual samples**

Confirmation testing is performed only on individual samples, not pooled samples.

In the context of NF VALIDATION™ Certification, all individual samples identified as positive by the Pathatrix™ Auto Salmonella spp. Kit Linked to Selective Agar Detection method must be confirmed by one of the following tests:

- By using the conventional tests described in the methods standardized by CEN or ISO (including the purification step). The confirmation step must start from the enrichment broth (either BPW or UHT milk).

- By performing a confirmatory test on observed characteristic colonies from the plated unlysed Pathatrix™ beads from the individual retested samples (“Retest individual samples after a positive pooled sample result” on page 28), as described in the following table.

<table>
<thead>
<tr>
<th>Characteristic colonies observed</th>
<th>Confirmatory test</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLD agar</td>
<td>Oxoid™ Brilliance™ Salmonella Agar</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

- Using any other method certified by NF VALIDATION™; the principle must be different from that of the Pathatrix™ Auto Salmonella spp. Kit Linked to MicroSEQ™ Salmonella spp. Detection Kit method (immunomagnetic separation and PCR). The detection protocol of the second validated method used for the confirmation shall be followed entirely. All steps which are before the step from which the confirmation is started shall be common to both methods (that is, enrichment in BPW at 37°C).
In the event of discordant results (positive with the alternative method, non-confirmed by one of the means described above, and in particular the latex test), the laboratory must follow the necessary steps to ensure the validity of the result obtained.

**Note:** Some strains of *Salmonella* belonging to the serovar Dublin may show a weak to nil magenta pigmentation, because of their low esterase activity.
## General troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| 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™ 10 Pooling Kit (Cat. No. POOL1010MLN) when sampling curdled enriched cultures. |
| 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 | Carryover contamination occurred. | 1. Repeat the assay using fresh aliquots of all reagents 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. |
<p>| 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. |</p>
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| In negative control wells, no IPC signal is detected, but a target-specific signal is detected | 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 the sample 1:5 with Nuclease-free Water to dilute PCR inhibitors, and repeat the assay. 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 model). | 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. |
<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| 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.

   ![Amplification Plot Diagram]

   - Refer to “Interpretation of the amplification plot for samples with a Result Warning” on page 35.

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).

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPC curve <strong>does not</strong> cross the threshold line.</td>
<td>IPC in this sample was inhibited.</td>
<td>Add 5 µL the stored DNA sample lysate to 25 µL of Nuclease-free Water and repeat the PCR.</td>
</tr>
<tr>
<td>IPC and Target curves <strong>do not</strong> cross the threshold line.</td>
<td>PCR inhibition in the sample.</td>
<td>Add 5 µL the stored DNA sample lysate to 25 µL of Nuclease-free Water and repeat the PCR.</td>
</tr>
<tr>
<td>IPC curve <strong>does</strong> cross the threshold line and target curve <strong>does not</strong> cross the threshold line.</td>
<td>No PCR inhibition. Sample is negative for target detection.</td>
<td>None needed; result is negative.</td>
</tr>
</tbody>
</table>
Go to thermofisher.com/foodsafety for a list of certified workflows for detection of Salmonella spp.

**Table 8** NF VALIDATION™ Certification of the workflow

<table>
<thead>
<tr>
<th>Certification</th>
<th>Expiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For information about the expiration date of the NF VALIDATION™ certifications, refer to the certificates, ABI 29/06-11/13 and ABI 29/07-11/13, available at nf-validation.afnor.org/en or at thermofisher.com[1].</td>
</tr>
</tbody>
</table>

[1] In the Product Literature section of the product web page.

The following methods have been certified by “NF VALIDATION™”:

- Pathatrix™ Auto *Salmonella* spp. Kit Linked to Selective Agar Detection (Certificate ABI 29/06-11/13)

The NF VALIDATION™ study was carried out according to the EN ISO 16140-2 standard against the reference method detailed in EN ISO 6579-1.

The validated workflow with PCR detection described in this user guide includes:

- Enrichment in BPW
- Pathatrix™ 10-Pooling Salmonella spp. Kit
- Pathatrix™ Salmonella spp. Kit
- MicroSEQ™ Salmonella spp. Detection Kit
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- RapidFinder™ Express Software, Version 1.1 or higher
- Confirmation testing as described in “Confirmation testing of PCR-positive individual samples” on page 27
The validated workflow with selective agar detection described in this user guide includes:

- Enrichment in BPW
- Pathatrix™ 10-Pooling Salmonella spp. Kit
- Pathatrix™ Salmonella spp. Kit
- Selective agar plating on XLD and Oxoid™ Brilliance™ Salmonella Agar
- Confirmation testing as described in “Confirmation testing of positive individual samples” on page 29

The workflows have been certified for use with the matrices described in the following table.

**Note:** Samples may be pooled only with samples of the same matrix type within a category.

### Table 9  Validated matrices

<table>
<thead>
<tr>
<th>Reference method</th>
<th>Category</th>
<th>Type</th>
<th>Example food items</th>
</tr>
</thead>
</table>
| EN ISO 6579-1    | Heat-treated milk and dairy products    | Sterilized or pasteurized milk and dairy products (UHT, canned or pasteurized) | • Fermented/acidified milks and yogurts  
• Milks, desserts, ice cream  
• Pasteurized cheeses, creams, butters  

Dry                                                                 | • Milk powders  
• Powders for dairy desserts  
• Infant formula with and without probiotic  

Raw beef meats (fresh and frozen, seasoned or not)                     | Fresh raw beef meats  
Frozen raw beef meats                                                   | Beef trim and ground beef  
Seasoned beef meats                                                     | Bolognaise, carpaccios, tartars, etc  

Cocoa and cocoa products                                              | Cocoa powders  
Cocoa-based products and chocolates                                     | Cocoa powders  
Raw materials                                                           | Cocoa beans  

General remarks and recommendations:

- Comply with Good Laboratory Practices (GLP; refer to EN ISO 7218 standard and EN ISO 22174).
- In the context of NF VALIDATION™ Certification, samples of more than 25 grams have not been tested.
Good laboratory practices for PCR

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.
  – Amplication 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 DNAZap™ Solutions (Cat. No. AM9890).

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, see the “Documentation and Support” section in this document.
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.

**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! 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.

Documentation and support

Food Safety support

Website: thermoscientific.com/foodmicro 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, and select the appropriate country from the dropdown menu.

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Publication number</th>
</tr>
</thead>
<tbody>
<tr>
<td>RapidFinder™ Express Software Quick Reference</td>
<td>4480999</td>
</tr>
<tr>
<td>Thermo Scientific™ KingFisher™ Flex User Manual</td>
<td>N07669</td>
</tr>
<tr>
<td>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</td>
<td>4378657</td>
</tr>
<tr>
<td>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</td>
<td>4387777</td>
</tr>
</tbody>
</table>

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

