Real-time PCR Detection of *Salmonella* spp. in Food and Environmental Samples

USER GUIDE

Using automated DNA isolation and magnetic bead-based technology

for use with:
PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing
KingFisher™ Flex Purification System with 96 Deep-Well Head
MagMAX™ Express-96 Deep Well Magnetic Particle Processor
MicroSEQ™ Salmonella spp. Detection Kit
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
RapidFinder™ Express Software v2.0 or later

Catalog Numbers 4428176, 4480466, 4403930
Publication Number 4405968
Revision G

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031001

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

Revision history: 4405968 G (English)

Revision	Date	Description
G	20 August 2024	Troubleshooting was added for possible instance of varying morphology of PCR pellets.
F	28 December 2021	• Instructions were added for enrichment of 375 g cocoa and chocolate-based samples. • The software version was updated for the RapidFinder™ Express Software. • Characteristics were added for the 7500 Fast Real-Time PCR Instrument.
E	1 March 2021	• The storage temperature for Magnetic Particles was changed from 4°C to room temperature to reflect new specifications. • The procedural guidelines were reorganized and made minor wording changes were made for clarification or to align with current template organization, style, and format. • The KingFisher™ Flex-96 instrument was added. • The content was updated with the Thermo Fisher Scientific template with associated updates to legal statements.
D	1 November 2013	• Instructions were added for PCR with the MicroSEQ™ Salmonella spp. Detection Kit and confirmation testing. • The document title was changed to reflect additional instructions. • Logos and certification details were added for AOAC *Performance Tested Methods*℠ certification. • Additional troubleshooting tips were added. • Instructions were clarified for handling Magnetic Particles, enriching samples in a chocolate matrix, and storage of DNA samples in the Elution Plate. • The number format was updated (time, temperature, and centrifugation speeds) for AOAC certification. • The contents were updated to a Life Technologies template with associated updates to the limited license information, warranty information, and trademark statement.
C	1 June 2010	A typographical error was corrected in the "Exclusions, Conditions, Exceptions, and Limitations" section.
B	1 September 2009	• Added instructions for handling Magnetic Particles, high-fat samples, and carryover of Magnetic Particles of food particulate residues in final eluates. • Updated limited license information, warranty information, and trademark statements.
A	1 January 2009	New document for the *Real-time PCR Detection of Salmonella spp. in Food and Environmental Samples User Guide (AOAC)* to isolate DNA from *Salmonella* spp.

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

Contents

1 Overview

This guide describes the following AOAC *Performance Tested Methods*℠ workflow for detection of *Salmonella* spp. in food and environmental samples:

1. Enrichment of the following sample types:
   - 25 g (or 25 mL) of food in Buffered Peptone Water (BPW)
   - Up to 375 g of feeding stuffs in Buffered Peptone Water (BPW)
   - Up to 375 g of cocoa and chocolate-based products in Buffered Peptone Water (BPW) or Non-Fat Dried Milk (NFDM) supplemented with Brilliant Green
2. Automated preparation of PCR-ready DNA using the PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing and the KingFisher™ Flex Purification System with 96 deep-well head.

   The KingFisher™ Flex instrument enables high-throughput sample processing in a 96-well format with minimal handling.
3. Real-time PCR detection of *Salmonella* spp. DNA using the MicroSEQ™ Salmonella spp. Detection Kit and RapidFinder™ Express Software on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.
4. Confirmation testing of positive samples.

See “AOAC *Performance Tested Methods*℠ Certification” on page 29 for detailed information about the AOAC certification. See “Specificity” on page 28 for details about assay specificity.

This workflow is intended for use by microbiological analysts who need to test for *Salmonella* spp. in food or environmental samples. These kits are for use in food and environmental testing only and not for any animal or human therapeutic or diagnostic use.

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

2 Enrich food or environmental samples

Materials for enrichment of food samples

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

Item	Source
Equipment	
Incubator, 30±1°C and 34°C to 38°C	MLS
Homogenizer Laboratory Blender	DB5000A or equivalent
Diluflux™ Pro Automated Gravimetric Dilutor	DB4150A, DB4100A, or equivalent
Consumables (for environmental samples only)	
Swab, sponge	MLS
15-mL conical tubes	MLS
Homogenizer bag appropriate for the sample type and size	
Homogenizer bag BagFilter™ 400	DB4011A or equivalent
Homogenizer bag BagPage™ 400	DB4012A or equivalent
Homogenizer bag BagLight™ 400	DB4013A or equivalent
Homogenizer bag RollBag™ 1300	DB4014A or equivalent
Reagents	
Buffered Peptone Water (BPW)	DF1049, CM1211B, or equivalent
Non-Fat Dried Milk (NFDM) (for cocoa and chocolate samples only) **Note:** According to EN ISO 6887-4:2017, for the cocoa and chocolate category, NFDM could be replaced with UHT Milk.	MLS
Brilliant Green dye solution (for cocoa and chocolate samples only)	MLS
Dey Engley (D/E) Neutralizing Broth (for environmental swabs or sponges)	R453042 or equivalent

Enrich samples

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

1. Prepare Buffered Peptone Water (BPW) according to the manufacturer's instructions.

   Note: For growth of bacteria in a cocoa or chocolate matrix, use reconstituted pre-warmed NFDM (100 g/L water; sterilized after reconstitution) or pre-warmed BPW as pre-enrichment broth. Ensure the addition of Brilliant Green (0.018 g/L) to NFDM for products with high background flora.

2. Combine BPW and sample as described in the following table.

Sample type [1]	Method
Food, 25 g or 25 mL	Add 225 mL of BPW to 25 g (or 25 mL) of sample.
Cocoa and chocolate products (up to 375 g)	Add 3,375 mL of pre-warmed NFDM to 375 g of sample or add 3,375 mL of pre-warmed BPW to 375 g sample. **Note:** The same 1:10 dilution ratio can be used for smaller sample sizes.
Feeding stuffs (up to 375 g)	Add 3,375 mL of BPW to 375 g of sample. **Note:** The same 1:10 dilution ratio can be used for smaller sample sizes.
Environmental swab	**1.** Prewet the swab with 0.5 mL of D/E Neutralizing Broth. **2.** Wipe the surface area to be tested. **3.** Add the swab to 10 mL of BPW in a 15-mL conical tube.
Environmental sponge	**1.** Prewet the sponge with 10 mL of D/E Neutralizing Broth. **2.** Wipe the surface area to be tested. **3.** Add the sponge to 100 mL of BPW.

[1] See table in "AOAC *Performance Tested Methods*℠ Certification" on page 29

3. Mix the sample with enrichment broth as described in the following table.

   A filtered bag can be used for enrichment of samples with particulates.

Sample type	Method
Food: • Coarse food types, such as meat, poultry, and seafood[1] • Eggs	Homogenize the sample thoroughly using a blender.
Food: Liquids or powdered	Shake the bag at least 25 times to achieve a homogeneous suspension.
Environmental swab	Twirl the swab for 90±30 seconds.
Environmental sponge	Use one of the following methods: • Use a nonfiltered, non-mesh enrichment bag and homogenize the sample thoroughly using a blender. • Hand squeeze for about 1 minute.

[1] Hand massage foods that cannot be processed in a homogenizer.

4. Incubate at 34–38°C under static conditions for 16–20 hours.

3 Isolate DNA with the PrepSEQ™ Nucleic Acid Extraction Kit

Product description

The PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing is designed for preparation of high-quality microbial nucleic acid from broth cultures. Magnetic beads allow efficient DNA capture and sample washing. The kit enables high-throughput automation in a 96-well plate format on the KingFisher™ Flex Purification System with 96 Deep-Well Head. Go to **thermofisher.com/foodsafety** for a list of workflows with non-automated DNA isolation methods.

Materials for DNA Isolation

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

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

Table 1 PrepSEQ™ Nucleic Acid Extraction Kit

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol)[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate[3]	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	

Table 1 PrepSEQ Nucleic Acid Extraction Kit *(continued)*

Contents	Cat. No. 4480466 (100 reactions)	Cat. No. 4428176 (300 reactions)	Storage[1]
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	15°C to 30°C
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	–25°C to –15°C

[1] See the expiration date on the box.
[2] Add ~35 mL of 100% isopropanol to the empty bottle before use.
[3] Add 74 mL of 95% ethanol before use.

Table 2 Materials required for automated isolation

Item	Source
KingFisher™ Flex Purification System with 96 Deep-Well Head[1]	A32681, 96 deep-well plate, or equivalent[2]
KingFisher™ Flex Microtiter Deep-Well 96 plate, v-bottom	95040450
KingFisher™ 96 KF microplates (200 µL)	97002540
KingFisher™ Flex 96 heating block	24075420
KingFisher™ 96 tip comb for deep-well magnets	97002534
Finntip™ Filtered Pipette Tips	94052320 or equivalent

[1] MagMAX™ Express-96 Magnetic Particle Processor may be used as an alternative.
[2] For the KingFisher™ Flex instrument, 96 plate with standard magnetic head (Cat. No. 5400620), the 96 deep-well magnetic head is required (Cat. No. 24074430).

Table 3 Other materials not included in the PrepSEQ™ Nucleic Acid Extraction Kit

Item	Source
Equipment	
Benchtop microcentrifuge	Eppendorf™ 5415 D or equivalent
96-Well Magnetic-Ring Stand	AM10050
Block heater, 37°C	MLS
Laboratory mixer (vortex or equivalent)	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
(Optional, but recommended) Plate centrifuge	MLS
Consumables	
Disposable gloves	MLS

Table 3 Other materials not included in the PrepSEQ Nucleic Acid Extraction Kit *(continued)*

Item	Source
Micropipette tips, aerosol-resistant	MLS
(Optional) MicroAmp™ Clear Adhesive Film	4306311
Microcentrifuge tubes, PCR clean, 1.5-mL	MLS
Reagents	
Ethanol, 95%	MLS
Isopropanol, 100%	MLS
Nuclease-Free Water (not DEPC-Treated)	AM9938

Procedure overview

The enriched sample (1 mL) is centrifuged. If the enriched sample produces a large food pellet, a fresh aliquot of the enriched sample is processed using the preclarification protocol. The sample pellet is lysed and processed on the instrument. Then, Binding Solution and Magnetic Particles are added to the samples, and after washing by the instrument, nucleic acid is eluted with 140 µL of Elution Buffer. The eluted DNA sample is ready for downstream PCR.

Workflow

Centrifuge sample after enrichment

Centrifuge 1 mL of sample at 12, 000–16,000 × *g* for about 3 minutes.

▼

If no large pellet: remove and discard the supernatant.

▼

If a large pellet: perform the preclarification protocol on a fresh, 1-mL aliquot of the enriched sample.

▼

Set up the Lysis Plate

Resuspend pellet in 300 µL of Lysis Buffer.

▼

Transfer the sample to a Deep Well Plate (Lysis Plate).

▼

Set up the processing plates

Tip Comb plate: Standard plate + Deep Well Tip Comb

Elution Plate: 140 µL Elution Buffer

Wash Plate 1: 300 µL Wash Buffer

Wash Plate 2: 300 µL Wash Buffer

▼

Process samples on the instrument

Select a program, select **Start**, and load processing plates into the instrument.

▼

After 18 minutes, add 30 µL of Magnetic Particles and 180 µL of Binding Solution. Reload the plate, and press **Start**.

▼

When the run is complete, DNA is in Elution Buffer (Elution Plate).

▼

Proceed to PCR, or seal the plate and store the DNA below –18°C.

Before first use of the kit

Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, then mix well. Label the bottle to indicate that ethanol is added.

Before each use of the kit

Resuspend Magnetic Particles

IMPORTANT! Mix the particles vigorously before each use to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

1. Incubate the tube of Magnetic Particles at 37±1°C for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

   Note: If the white precipitate is not completely dissolved after 10 minutes at 37°C, apply longer incubation times and higher temperatures (up to 50°C).

3. Keep at room temperature (23±5°C) until ready for use.

Centrifuge the enriched culture

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

1. Transfer 1 mL of enriched culture to a 1.5-mL microcentrifuge tube.
2. Centrifuge the tube at 12,000–16,000 × *g* for about 3 minutes.

   (Optional) If the sample creates a large pellet, follow "For large sample pellets: perform the preclarification protocol" on page 14 with a fresh sample of the enriched culture.
3. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.

   (Optional) If necessary, follow "For high-fat samples: remove fat layer before lysis" on page 14.

For large sample pellets: perform the preclarification protocol

For samples that produce a large pellet upon initial centrifugation, follow this preclarification protocol:

1. Transfer a fresh, 1 mL sample of enriched culture to a 1.5-mL microcentrifuge tube.
2. Centrifuge the tube containing the sample at about 4000 × *g* for about 1 minute.
3. Transfer the supernatant to a new 1.5-mL microcentrifuge tube without disturbing the pellet. Discard the pellet.
4. Centrifuge the tube containing the supernatant at 12,000–16,000 × *g* for about 3 minutes.
5. Remove and discard the supernatant as quickly as possible to prevent dissipation of pellet.
   (Optional) If necessary, follow "For high-fat samples: remove fat layer before lysis" on page 14.

For high-fat samples: remove fat layer before lysis

For samples that contain a distinct, top, fat layer following centrifugation, remove the fat layer and supernatant as follows:

Type of fat layer	Fat layer and supernatant removal
Liquid	1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet. 2. Continue to collect supernatant from the top surface until all the supernatant is removed. 3. Discard the supernatant into a waste container.
Solid	1. Use a pipette tip to gently dislodge the fat layer without disturbing the pellet. 2. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed. 3. Discard the supernatant into a waste container.

Set up the Lysis Plate

1. Add 300 µL of the Lysis Buffer to the tube.
2. Resuspend by pipetting up and down.
   Alternatively, vortex until the pellet is resuspended, and quick spin for about 5 seconds to remove the Lysis Buffer from the tube lid.
3. Transfer the sample to a deep well Lysis Plate.
4. (Optional but recommended) Set up a negative extraction control (NEC) containing 300 µL of Lysis Buffer in the Lysis Plate.

Set up the processing plates

Set up the processing plates as described in the following table.

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well deep-well tip comb in a standard plate.
Elution Plate	Standard	Add 140 µL of Elution Buffer to each sample and control well.
Wash Plate 1	Deep-Well	Add 300 µL of Wash Buffer to each sample and control well.
Wash Plate 2	Deep-Well	Add 300 µL of Wash Buffer to each sample and control well.

Process samples on the instrument

1. Power on the instrument, select the program, and press **Start**.

Instrument	Program
KingFisher™ Flex	**4412639PrepSEQ_Sal**

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the tip comb, then press **Start**.
Elution Plate	Load the Elution Plate, then press **Start**.
Wash Plate 2	Load Wash Plate 2, then press **Start**.
Wash Plate 1	Load Wash Plate 1, then press **Start**.
Lysis Plate	Load Lysis Plate, then press **Start**.

3. Dispense the Magnetic Particles and Binding Solution when prompted by the instrument (after ~18 minutes).
   a. Vortex the Magnetic Particles for about 10 seconds until resuspension is complete.
   b. Add 30 µL of Magnetic Particles to each well.
   c. Add 180 µL of Binding Solution to each well.
   d. Load the plate back into the instrument. Press **Start**.
4. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.
   The Elution Plate contains DNA in Elution Buffer.
5. Proceed directly to real-time PCR, or store the DNA in one of the following ways:
   - 5±3°C for up to 24 hours.
   - Below –18°C for long-term storage.

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

Product description

The MicroSEQ™ Salmonella spp. Detection Kit detects *Salmonella* spp. simply, reliably, and rapidly in food samples using a lyophilized reagent format. The assay uses the polymerase chain reaction (PCR) to amplify a unique microorganism-specific DNA target sequence and a TaqMan™ probe to detect the amplified sequence.

The MicroSEQ™ assay beads contain all the components necessary for the real-time PCR reaction: *Salmonella* spp.-specific probe and primers, enzyme, and other buffer components. The assay beads also contain an internal positive control (IPC) probe, primers, and template, to monitor for PCR inhibition. Pathogen Detection Negative Control is included in the kit. Unknown samples and positive control samples are provided by the investigator.

RapidFinder™ Express Software provides step-by-step instructions to set up the real-time PCR assays followed by automated data analysis. Online help is provided within the software. The software is designed for use on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.

Materials for PCR detection

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

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

Table 4 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.

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

(continued)

Item	Source
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
Reagents	
Nuclease-Free Water (not DEPC-Treated)	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.

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

- If DNA samples were stored before PCR, thaw (if necessary), vortex, then centrifuge at 1,000–2,000 × *g* for approximately 1 minute to remove any condensation from the adhesive film before opening the plate (to avoid cross contamination).
- 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 30.

Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)

If you see this in the Elution Plate...	Do this...
Oil droplets as a top layer	After lysis, food samples with high fat or oil content can form a top layer containing fat and debris over the aqueous phase containing the DNA. Collect the DNA sample for PCR from the clear middle phase, avoiding the top layer and bottom pellet (See Figure 1).
Magnetic Particles	1. Place the Elution Plate on a 96-well magnetic ring stand for at least 1 minute. 2. Collect the eluate for PCR while the Elution Plate remains on the magnetic stand. Avoid touching the Magnetic Particles.
Particulate residue from food sample	If the particulate residue is not removed using a 96-well magnetic ring stand: 1. Centrifuge the Elution Plates at about 4000 × *g* for about 30 seconds in a plate centrifuge. 2. Avoid the particulate residue, and collect eluate for PCR.

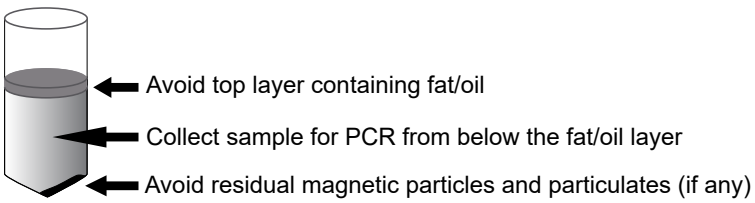

Figure 1 High-fat samples: Collect sample from middle phase after lysis.

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.

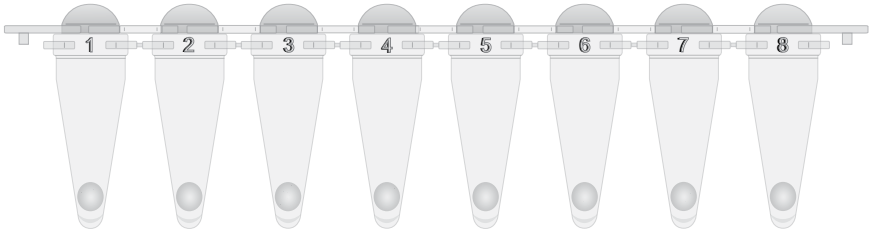

Figure 2 MicroAmp™ Tube Strip labeling The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.

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

On the main page of the RapidFinder™ Express Software, select **Create/Edit a Run File**, then select 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. If necessary, thaw samples and controls completely, and mix each sample or control thoroughly.

   If the Elution Plate contains oil droplets, magnetic particles, or food particulate residue, see “Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)” on page 19.

   If the DNA samples have been stored, see “Sample handling” on page 19.

2. Following the layout determined by RapidFinder™ Express Software, add 30 µL of sample or control to each assay bead at room temperature (23±5°C), and mix by gently pipetting up and down a few times.

   Beads dissolve in 1–5 seconds.

   Alternatively, vortex the assay tubes after they are capped in the final step.

3. Seal the tubes with the transparent, optical cap strips provided in the kit.

4. Ensure that the reactions are thoroughly mixed: if reactions were not previously mixed during the pipetting step, vortex at high speed for 5–10 seconds.

5. Ensure that the reagents are at the bottom of tubes: briefly centrifuge the tube strips at 200–600 × *g* for about 20 seconds.

   IMPORTANT! If needed, repeat the vortex/centrifugation steps to ensure complete mixing of the samples with the assay beads.

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 3).

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

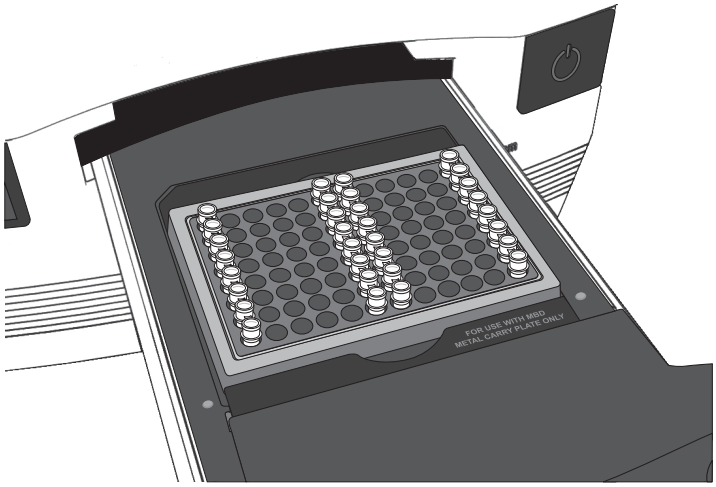

Figure 3 7500 Fast instrument tube layout RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby 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.

If necessary, investigate results in SDS Software

Follow the RapidFinder™ Express Software prompts for "Investigating Warning Results or Failed Runs in the 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.

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1. From **View Results** in the RapidFinder™ Express Software, select and open the run file, and then click **View in SDS**.

2. Select **File ▸ Save As**, and save the run file under a new name.

5 Recommended confirmation methods

In the context of AOAC *Performance Tested Methods*℠ certification, samples with presumptive PCR positive results should be tested with the appropriate reference method for the sample matrix (See "AOAC *Performance Tested Methods*℠ Certification" on page 29 for detailed information.)

Confirm well-isolated, presumptive positive *Salmonella* colonies using an appropriate Official Method of Analysis of AOAC International (AOAC-OMA) or EN ISO 16140-6:2019 validated confirmation method, or any appropriate reference confirmation procedure (e.g. FDA/BAM Chapter 5, USDA/FSIS MLG 4.10, EN ISO 6579:2017 standard) depending on your regional legislation.

In the event of a positive PCR result, which cannot be confirmed using the steps described above, all necessary measures must be taken by the laboratory to establish the true status of the sample before reporting the result.

A Troubleshooting

Observation	Possible cause	Recommended action
A visual difference in PCR beads is observed.	PCR pellets can exhibit differences in morphology.	Ensure thorough pipette mixing followed by vortexing on high speed to confirm pellet is in solution. After PCR, if IPC failure is observed, repeat the reaction.
Bacterial pellet is difficult to avoid during removal of supernatant	The sample was left unattended before removal of the supernatant, causing dissipation of the bacterial pellet.	Remove the supernatant immediately following centrifugation.
	The size of the bacterial pellet is very small and difficult to see.	Remove the supernatant carefully, leaving behind up to 50 µL of supernatant to avoid aspiration of the pellet.
Bacterial pellet is difficult to resuspend during lysis	Pellet is too hard.	Ensure maximum resuspension of the pellet in the Lysis Buffer or Proteinase K Buffer Mix before proceeding.
		Transfer the entire contents, including the incompletely resuspended pellet (if any) to the Lysis Plate.
		Follow the PrepSEQ™ Preclarification Protocol (“For large sample pellets: perform the preclarification protocol” on page 14).
Inhibition of downstream PCR, indicated by nondetection of IPC reaction	Magnetic Particles were in the Elution Plate.	Avoid disturbing the Magnetic Particles during transfer of eluted DNA to the lyophilized assay. Avoid transfer of Magnetic Particles using one of the following methods *(optional)*: • Place the Elution Plate on the 96-Well Magnetic Ring Stand during transfer of eluted DNA sample to the lyophilized assay. • Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 × *g* for approximately 30 seconds to pellet the Magnetic Particles to the bottom of the plate.

Observation	Possible cause	Recommended action
Inhibition of downstream PCR, indicated by nondetection of IPC reaction *(continued)*	Elution Plate contained incompletely removed particulate residue from the food sample.	Avoid residue during transfer of eluted DNA to the lyophilized assay. *(Optional)* Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 × *g* for approximately 30 seconds to pellet the food residue to the bottom of the plate.
	For samples of enriched culture that were centrifuged before lysis, the removal of sample supernatant before addition of lysis buffer was incomplete.	Ensure maximal removal of the supernatant without disturbing the bacterial pellet.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the 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 pipette the positive control into all positive control wells.
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. Additionally, no IPC signal in negative control wells could be caused by: • A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 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 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 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, then repeat the assay. If PCR remains inhibited, repeat the sample preparation. Refer to other troubleshooting suggestions for removal of Magnetic Particles or particulate residue from the DNA sample.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the 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 occurred.	After adding 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, then centrifuge the strips at 200–600 × *g* for about 10 seconds. 2. Vortex the strips again on high speed for about 10 seconds, then 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 did 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, then 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.

B

Supplemental information

Sensitivity

The sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The AOAC *Performance Tested Methods*℠ workflow described in this user guide allows you to detect 1 to 3 colony-forming units (CFU) in tested samples of food or environmental swab and sponge samples. Following the workflow described in this user guide, the limit of detection is 10^3 CFU/mL (not part of AOAC validation study).

Specificity

The MicroSEQ™ Salmonella spp. Detection Kit can detect all *Salmonella enterica* serovars tested and did not detect any non-*Salmonella* species tested. The genus *Salmonella* consists of the two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* incorporates the most important clinical serovars for humans. The method does not allow detection of *Salmonella bongori*.

Operational conditions

Table 5 Magnetic Particle Processor operation 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

Table 6 Applied Biosystems™ 7500 Fast Real-Time PCR Instrument operation 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

AOAC *Performance Tested Methods*℠ Certification

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

Table 7 *Performance Tested Methods*℠ Certification of the workflow

Certification	
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 PrepSEQ™ kits and 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 media: Buffered Peptone Water and Non-Fat Dried Milk supplemented with Brilliant Green
- PrepSEQ™ Nucleic Acid Extraction Kit
- MicroSEQ™ Salmonella spp. Detection Kit
- Confirmation testing of positive samples
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 8 for characteristics) with RapidFinder™ Express Software v2.0 or later.

Table 8 7500 Fast Real-Time PCR Instrument characteristics

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

Reference method	Matrix
EN ISO 6579:2017	**Food (25 g sample size)**: raw ground beef, raw chicken wings, raw shrimp, cantaloupe, brie, dry infant formula, chocolate, dry pet food, shell eggs, black pepper
USFDA BAM, Chapter 5	**Food (25 g sample size)**: peanut butter **Pet food (up to 375 g sample size)**: dry pet food **Cocoa and chocolate products (up to 375 g sample size)**: cocoa powder, cocoa butter, cocoa liquor, dark chocolate (> 70% cocoa) **Environmental samples**: stainless steel, sealed concrete, plastic, ceramic tile, rubber

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**.

C

Safety

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

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

Chemical safety

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

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

Biological hazard safety

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

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

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

D

Documentation and support

Food safety support

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

Support email:

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

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

Customer and technical support

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

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

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

Related documentation

Document	Publication number
RapidFinder™ Express Software Quick Reference	4480999
Thermo Scientific™ KingFisher™ Flex User Manual	N07669

(continued)

Document	Publication number
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
PCR Starter Kit for 96-well blocks, 0.2 mL, User Guide	A24829

References

AOAC INTERNATIONAL Guidelines, Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, version 2012.

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.

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

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

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

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

EN ISO 6887-5:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products.

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

EN ISO 16140-6:2016. Microbiology of food and animal feed – Method validation – Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

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

FDA Bacteriological Analytical Manual (BAM), Chapter 5 – Salmonella spp.

USDA/FSIS Microbiology Laboratory Guidebook, Revision 4.10 – Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.

U.S. Food and Drug Administration, Bacteriological Analytical Manual (BAM), Chapter 5; go to **https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella**. Accessed 23 Sept. 2013.