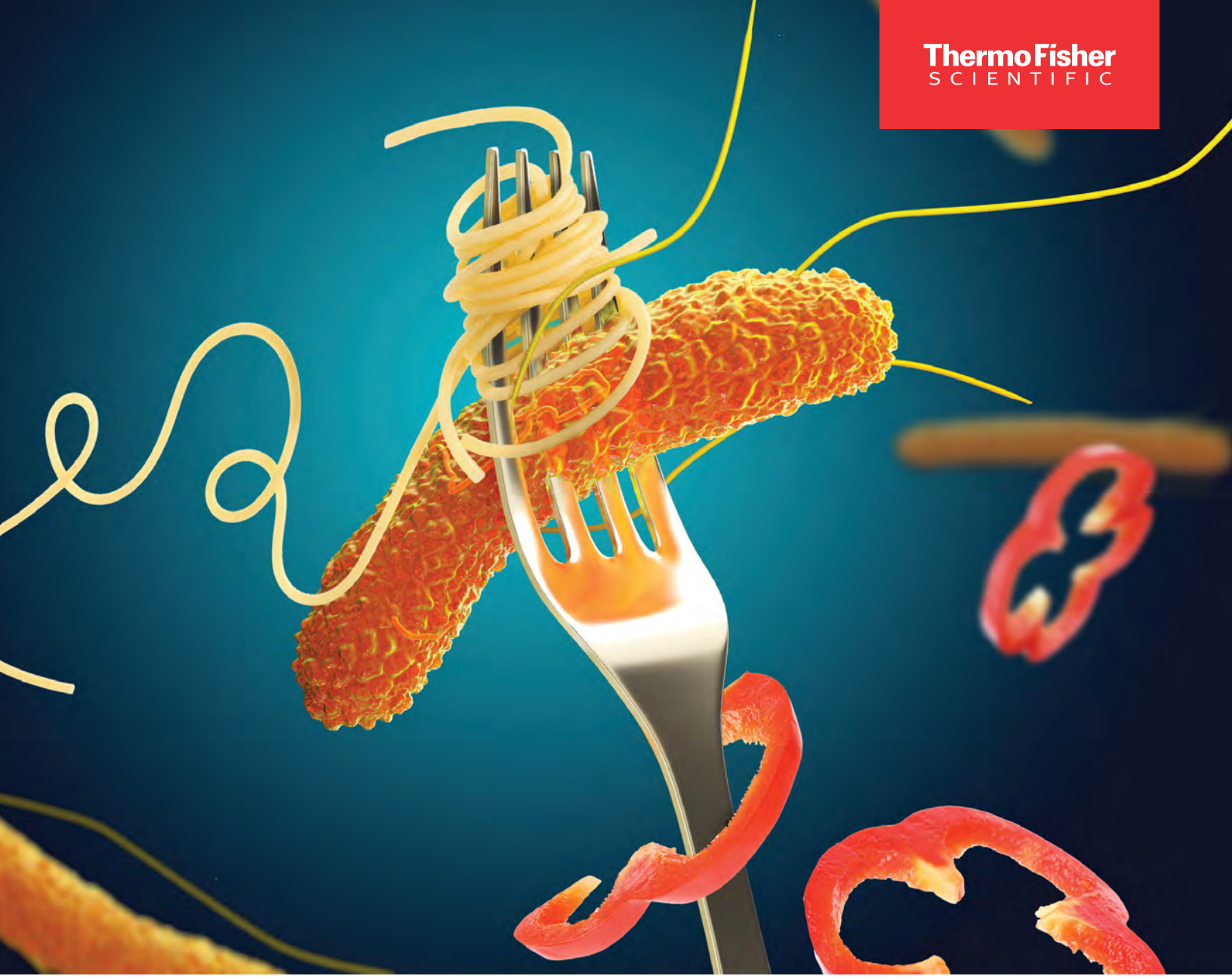
ThermoFisher SCIENTIFIC



Food safety testing

Food Microbiology Testing Methods: *Salmonella* species

thermoscientific

Salmonella species

The genus *Salmonella* belongs to the family Enterobacteriaceae. *Salmonella* are Gram-negative, non-spore forming rods. There are over 2,500 serovars of *Salmonella*, which are characterized according to somatic (O) and flagellar (H) antigens.

Salmonellae are chemoorganotropic, with an ability to metabolize nutrients by both respiratory and fermentative pathways. The bacteria are oxidase and catalase negative, grow optimally at 37°C, and catabolize D-glucose and other carbohydrates with the production of acid and gas. However, they readily adapt to extreme environmental conditions and can grow at both elevated and refrigerated temperatures.

Incidence

Salmonellosis is the most frequently reported cause of foodborne illness. An estimated one million cases occur annually in the United States; of these, approximately 35,000 are laboratory-confirmed cases reported to the Centers for Disease Control and Prevention.

Infective dose

The ingestion of fewer than 1000 cells can constitute a human infectious dose. Lower cell numbers can be highly infectious, especially in high fat foods where the microorganism can escape the gastric acidity and be released in the intestine through bile mediated dispersion of the lipids.

Foods affected

Despite the general perception that chicken and egg products are the primary source of *Salmonella* infections, many outbreaks in recent years have been associated with tomatoes, peanut butter, and vegetable sprouts. Other affected foods include: raw meat, powdered infant formula, milk and dairy products, fish, cake mixes and chocolate, as well as pet food and animal feed.

The detection of *Salmonella* in foods before they are consumed is vital for safeguarding public health, and essential for preserving the financial health and reputation of food businesses.

Methods overview

The following methods described for *Salmonella* species include reference and alternative validated and certified methods as follows:

1. Reference Method: 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.
2. Reference Method: USDA FSIS MLG 4 - Isolation and Identification of *Salmonella* - Rapid Screening *Salmonella* Test Procedure
3. Reference Method: FDA BAM Chapter 5 - *Salmonella* Isolation Method
4. Alternative Method: Thermo Scientific™ SureTect™ Salmonella species PCR Assay Workflow
5. Alternative Method: Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Workflow
6. Alternative Method: Thermo Scientific™ SureCount™ Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Workflow
7. Alternative Method: Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay Workflow
8. Alternative Method: Thermo Scientific™ Salmonella Precis™ Method

References

1. United States Food and Drug Administration. Bad Bug Book, 2nd Edition: Foodborne pathogenic microorganisms and natural toxins handbook: Salmonella: https://www.fda.gov/food/foodborne-pathogens/bad-bug-book-second-edition
2. D'Aoust J, Maurer J. 2007. Salmonella Species, p 187-236. In Doyle M, Beuchat L (ed), Food Microbiology: Fundamentals and Frontiers, Third Edition. ASM Press, Washington, DC.

Reference Method: ISO 6579:2017 Microbiology of the food chain

Horizontal method for the detection, enumeration and serotyping of *Salmonella*

Part 1: Detection of *Salmonella* spp.

This is a summary of ISO 6579:2017 Part 1: Detection of *Salmonella* spp. in food (including milk and milk products), in animal feed, in animal faeces, and in environmental samples from the primary production stage.

Workflow overview for ISO 6579:2017 Part 1

Day 0

25 g + 225 mL Buffered Peptone Water (BPW), 1 swab + 10 mL BPW, 1 sponge + 100 mL BPW, 1 wipe + 225 mL BPW

Incubate for 16-20 h at 34-38°C

Day 1

Add 100 µL of primary enrichment to 10 mL of Rappaport-Vassiliadis Peptone Soya Broth (RVS) or Modified Semi-solid Rappaport Vassiliadis (MRSV) agar

Incubate for 21-27 h at 40.5-42.5°C

Add 100 µL of primary enrichment broth to 10 mL of Muller-Kauffman Tetrathionate Broth (MKTTn) supplemented with Novobiocin and Iodine-iodide

Incubate for 21-27 h at 34-38°C

Day 2

Plate 10 µL of secondary enrichment broth onto Xylose Lysine Deoxycholate Agar (XLD) and a secondary isolation medium
and
Plate 0.1 µL from MSRV or RVS onto XLD and a secondary isolation medium

Incubate XLD plates for 21-27 h at 34-38°C
(incubate the secondary isolation medium according to the manufacturer's instructions)

Day 3

Confirm up to five presumptive-positive colonies by selecting either well isolated colonies where there is sufficient growth, or by first sub-culturing on to a non-selective agar such as Nutrient Agar.

Incubate for 21-27 h at 34-38°C

Day 4-5

Biochemical confirmation
Refer to ISO standard for method details.

Acid and gas production: Triple Sugar Iron Agar (TSI)
Detection of urease: Urea Agar
Detection of β-galactosidase: L-Lysine Decarboxylation
Indole reaction: Tryptone Water, Kovac's Reagent

Serological confirmation
Refer to ISO standard for method details.

Detection of O, Vi, and H antigens:
Salmonella O Polyvalent Agglutinating Sera
Salmonella Vi Polyvalent Agglutinating Sera
Salmonella H Polyvalent Agglutinating Sera

Product order information for testing according to ISO 6579:2017 Part 1: Detection of *Salmonella* spp.

	Product description		Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water (ISO) (BPW)		500 g, makes 25 L	CM1049B
	RVS Broth		500 g, makes 18.7 L	CM0866B
	MSRV Agar (ISO)	MRSV Agar (ISO) Base	500 g, makes 15.8 L	CM1112B
	PrecisBlue Supplement		80 x 25 g samples	SR0259A
	Novobiocin Supplement	Novobiocin Selective Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Selective Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	MKTTn Broth	MKTTn Broth Base	500 g, makes 6.1 L	CM1048B
	XLD Agar		500 g, makes 9.4 L	CM0469B
	Brilliance™ Salmonella Agar	*Brilliance* Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Brilliance Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
	Nutrient Agar		500 g, makes 17.8 L	CM0003B
	TSI Agar		500 g, makes 7.7 L	CM0277B
	Urea Agar	Urea Agar Base	500 g, makes 20.8 L	CM0053B
		40% Urea Solution	10 vials, each for 100 mL	SR0020K
	Tryptone Water		500 g, makes 33.3 L	CM0087B
Thermo Scientific™ Oxoid™ Kovac's Reagent			10 mL bottle	MB0209A
Thermo Scientific™ Remel™ Salmonella O Polyvalent Agglutinating Sera (group A - S)			2 mL vial	R30858201
Thermo Scientific™ Remel™ Salmonella O/Vi Polyvalent Agglutinating Sera			2 mL vial	R30957401
Thermo Scientific™ Remel™ Salmonella H Polyvalent Agglutinating Sera			2 mL vial	R30858501

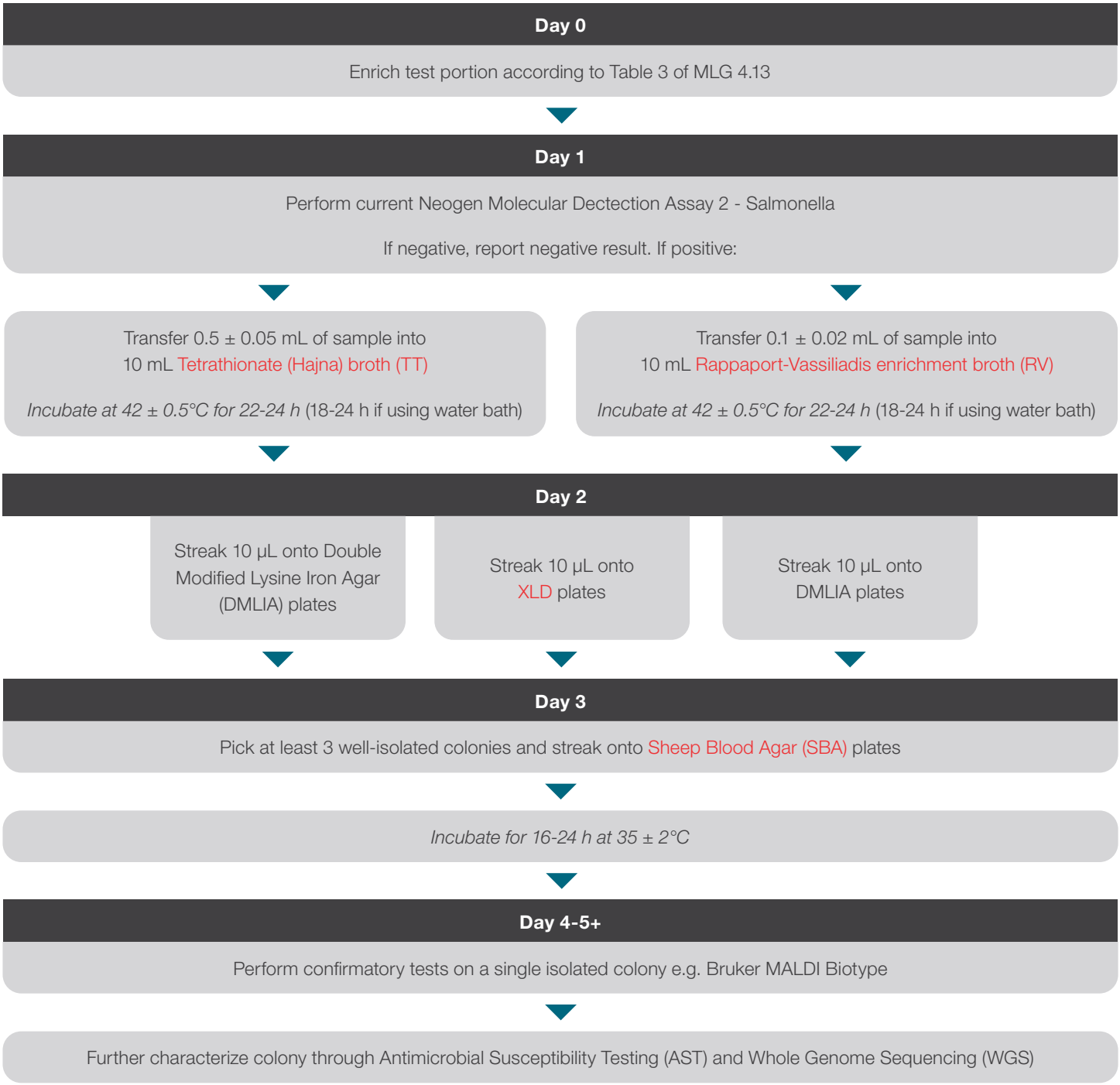
Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Reference Method: USDA FSIS MLG 4.15 - Isolation and Identification of *Salmonella*

Rapid Screening *Salmonella* Test Procedure

This is a summary of the Rapid Screening *Salmonella* Test Procedure from USDA FSIS MLG 4.15 - Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, Carcass, and Environmental Sponges.

Workflow overview for USDA FSIS MLG 4.15 - Isolation and Identification of *Salmonella* - Rapid Screening Salmonella Test Procedure



Product order information for testing according to USDA FSIS MLG 4 - Isolation and Identification of *Salmonella* - Rapid Screening *Salmonella* Procedure

	Product description	Format	Order code
Thermo Scientific™ Culture Media	Remel™ TT Broth	500 g, makes 10.9 L	CM0671B
	Oxoid™ Buffered Peptone Water (ISO)	500 g, makes 25 L	CM1049B
	Oxoid™ mTSB Broth	500g, makes 12.5 L	CM0989B
	Oxoid™ RV Broth	500 g, makes 16.7 L	CM0669B
	Remel™ XLD Agar	500 g, makes 9 L	CM0263B
	Oxoid™ BSA Agar	500 g, makes 12.5 L	CM0854B

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Reference Method: FDA BAM Chapter 5 - *Salmonella* Isolation Method

This is a summary of the standard reference *Salmonella* isolation method cited in the U.S Food & Drug Administration Bacteriological Analytical Manual.

Workflow overview for FDA BAM Chapter 5 - *Salmonella* Isolation Method

Day 0

Enrich test portion according to Section C of FDA BAM Chapter 5 for 24 ± 2 h at 35 ± 2°C

Day 1

Following pre-enrichment, either FDA LAMP or qPCR method, may be used to screen *Salmonella* in 24 h pre-enrichment cultures before isolation of *Salmonella*

Samples that have negative rapid screening results will be reported as negative, and no further culture analysis will be required. Samples that have positive rapid screening results must be confirmed culturally

Report negative results

Transfer 0.1 mL mixture to 10 mL Rappaport-Vassiliadis (RV) medium and another 1 mL mixture to 10 mL TT broth

Foods with a high microbial load:
Incubate RV medium for 24 ± 2 h at 42 ± 0.2°C. Incubate TT broth for 24 ± 2 h at 43 ± 0.2°C

Foods with a low microbial load:
Incubate RV medium for 24 ± 2 h at 42 ± 0.2°C. Incubate TT broth 24 ± 2 h at 35 ± 2°C

Day 2

Plate 10 µL incubated TT broth on Bismuth Sulfite (BS) agar, Xylose Lysine Desoxycholate (XLD) agar, and Hektoen Enteric (HE) agar

Repeat with 10 µL of RV medium or SC broth on XLD, HE, and BS agars

Incubate plates for 24 ± 2 h at 35 ± 2°C

Day 3

Confirm 2 or more colonies of *Salmonella* from each selective agar plate after 24 ± 2 h incubation

Day 4

TSI and LIA biochemical and serological confirmation

Product order information for testing according to FDA BAM Chapter 5 - *Salmonella* Isolation Method

	Product description	Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	TT Broth	500 g, makes 10.9 L	CM0671B
	RV Broth	500 g, makes 16.7 L	CM0669B
	SC Broth	500 g	CM0699B
	BS Agar	500 g, makes 25 L	CM0201B
	HE Agar	500 g, for 6.6 L	CM0419B
	XLD Agar	500 g, makes 9.4 L	CM0469B

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Alternative Method:

Thermo Scientific™ SureTect™ Salmonella species PCR Assay

A rapid real-time PCR method for the enrichment, detection and confirmation of *Salmonella* species in food, feed products, and environmental samples:

- Validated according to AOAC International and the ISO 16140 2:2016 standard
- Streamlined and rapid workflow – 96-well runs can be prepared with just a few simple steps
- Single enrichment protocols for faster time-to-result and streamlined workflow
- Pre-dispensed reagents, reducing handling steps and risk of error
- Universal PCR conditions for detecting multiple targets in the same run
- Intuitive, user-friendly software, avoiding subjective interpretation
- 'Plug and play' ready-to-use instruments out of the box
- Reduced time-to-result: 1 day compared with up to 5 days for reference methods

Enrichment
Single enrichment step

Sample preparation and lysis
Manual preparation with direct lysis in the Applied Biosystems™ SimpliAmp™ Thermal Cycler, or fully automated lysis and PCR preparation using the CyBio™ FeliX instrument as part of the Thermo Scientific™ SureTect™ Automation Workflow

Run and read
Results automatically interpreted

Report/confirm
Report PCR negative results and confirm PCR positive results after overnight incubation

Validation

The assay's workflows have been validated for a broad range of human foods, pet food, including large test portions, and environmental samples by AOAC OMA and ISO 16140-2:2016 according to NF VALIDATION™ by AFNOR Certification and by MicroVal™ Certification. The assay is also certified for primary production samples by NF Validation™ by AFNOR Certification.

AFNOR Certification validation certificate No. UNI 03/07-11/13 is available in PDF format from the AFNOR website https://nf-validation.afnor.org/en/food-industry/salmonella-spp/.

Workflow overview for SureTect Salmonella Species PCR Assay validated according to ISO 16140-part 2:2016 method

Day 0

Enrich test portion according to conditions stated for chosen matrix in the **user guide**. The assay has been tested on a broad range of human foods, pet food, including large test portions, and environmental samples.

Day 1

Add 10 µL of SureTect Proteinase K to each required SureTect Lysis Tube (supplied pre-filled with Lysis Reagent 1)

Add 10 µL enriched sample or diluted cocoa/chocolate sample to the SureTect Lysis Tube; add 10 µL sterile nuclease-free water to a SureTect Lysis Tube as a negative control

Incubate SureTect Lysis Tubes in the SimpliAmp Thermal Cycler at 36-38°C for 10 minutes followed by 94-96°C for 5 minutes, and 2 minutes at 9-11°C

Transfer 20 µL of lysate to SureTect PCR Tubes

Load SureTect PCR Tubes into the QuantStudio 5 PCR Instrument. Start PCR and review results at end of run

Report negative results

Day 1 (SureTect Automation Workflow)

Prepare the Sample Enrichment Tubes

Transfer between 700 µL to 1 mL of the enriched sample to each Sample Enrichment Tube

For chocolate and cocoa samples only, dilute the enrichment 1-in-5 (1 part enriched sample and 4 parts sterile BPW)

Prepare the Lysis tubes according to instructions. When prompted by the SureTect Automation Software, remove the caps of and the Proteinase K tubes and place the reagents in the correct deck position according to the layout. Then when prompted place the Sample Enrichment Tubes with the tube rack into the instrument

Prepare the PCR tubes according to instructions

After the lysis step is complete, the SureTect Automation Software will prompt to load the PCR tubes for the PCR sample preparation step. At this point, remove the seals

When complete, remove the PCR tubes. Start the PCR run with the QuantStudio 5 PCR Instrument. Review results at end of run

Day 2

Confirm PCR positive results by plating 10 µL of enrichment broth onto *Brilliance* Salmonella.
For samples with high background microflora first subculture in RVS Broth *(incubate for 21-27 h at 41.5°C)*

Depending on the legislation terrirtory, confirm presumptive-positive colonies with the Oxoid Salmonella Latex Kit, MicroBact GNB 24E or confirmatory tests described in ISO 6579-1:2017 method or with an ISO 16140 6:2019 or the FDA/BAM Chapter 5 confirmation tests or USDA/FSIS MLG 4.10 confirmation tests with an AOAC-OMA validated confirmation test

Product order information for SureTect Salmonella species PCR Assay workflow

Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water	Buffered Peptone Water (ISO)	500 g, makes 25 L	CM1049B
		Buffered Peptone Water (ISO-meat peptone)	500 g, makes 25 L	CM1211B
	PrecisBlue Supplement		1 vial, for 80 x 25 g samples	SR0259A
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	Vancomycin Supplement	Vancomycin Supplement - freeze-dried	10 vials of 3 mg	SR0186E
		Vancomycin Supplement - freeze-dried	10 vials of 5 mg	SR0247E
	ONE Broth Salmonella	ONE Broth Salmonella Base	500 g, makes 20 L	CM1091B
		ONE Broth Salmonella Supplement	10 vials, each for 225 mL	SR0242E
	Brilliance™ Salmonella Agar	*Brilliance*™ Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
	RVS Broth		500 g, makes 18.7 L	CM0866B
Thermo Scientific™ SureTect™ Salmonella species Assay (includes Proteinase K, Lysis Tubes (pre-filled with Lysis Reagent 1), Salmonella spp. PCR Tubes (pre-filled with Salmonella PCR tablets), Thermo Scientific 96-Well Pierceable Seals and PCR Tube caps)			96 tests	A56841
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A
Thermo Scientific™ Microbact™ GNB 24E			80 tests	MB1131A
Applied Biosystems™ SimpliAmp™ Thermal Cycler (Lysis step)			Instrument	A24811
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (includes Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or higher and laptop computer)			Instrument	A36328
Thermo Scientific™ SureTect™ Automation Platform, one of the following:		Automation Platform CHOICE Head	Instrument	A59017
		Automation Platform Head R 96	Instrument	A66295
Assurance Service Plan – additional 1-year warranty			Service agreement	SCQS5FSAT

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Alternative Method:

Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay

A rapid method for the enrichment, detection and confirmation of *Salmonella* species, *Salmonella* Typhimurium and *Salmonella* Enteritidis in raw pork and poultry, ready-to-eat and ready-to-reheat pork and poultry, shell eggs, production environment samples, and primary production samples (PPS):

- Validated according to ISO 16140 2:2016 standard
- First validated multiplex PCR assay for simultaneous detection of *Salmonella* species, and *Salmonella* serovars; Typhimurium and Enteritidis
- Designed as a tool specifically for *Salmonella* control programs in pork and poultry production
- Streamlined testing workflow and reduced waiting time for product release or intervention
- Reduced time to results as little as 16 hours compared with up to 5 days for standard culture methods
- Reduced time-to-result: 1 day compared with up to 5 days for the ISO reference method

Enrichment
Single enrichment step for meat and production environment samples

Sample preparation and lysis
Manual preparation with direct lysis in the SimpliAmp Thermal Cycler, or fully automated lysis and PCR preparation using the CyBio FeliX instrument as part of the SureTect Automation Workflow

Run and read
Results automatically interpreted

Report/confirm
Report PCR-negative results and confirm *Salmonella* spp., SE/ST results with single plate and serology

Validation

The SureTect Salmonella Multiplex Assay and SureTect Salmonella Multiplex Flex Assay methods have been validated and approved by AFNOR (ISO 16140-2:2016) and AOAC-PTM validated on a range of matrices.

NF VALIDATION certificate No. UNI 03/12-01/18 is available in PDF format from the AFNOR website https://nf-validation.afnor.org/en/food-industry/salmonella-spp/.

Workflow overview for SureTect Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay validated according to ISO 16140-part 2:2016 standard

Day 0

Raw and ready-to-eat pork and poultry samples: 25 g + 225 mL BPW (ISO-meat peptone) supplemented with 12 mg/L Novobiocin Supplement or PrecisBlue Supplement

40.5-42.5°C for 14-18 hours

Production environment samples: Add 25 g of production environment sample to 225 mL of BPW (ISO-meat peptone). Add 1 swab to 10 mL of BPW (ISO-meat peptone). Add 1 sponge to 100 mL of BPW (ISO-meat peptone). Add 1 wipe to 225 mL of BPW (ISO-meat peptone).

40.5-42.5°C for 14-18 hours

Day 1

Remove ~1.5 mL of enrichment to a sterile sealable tube

First, add 10 µL of Proteinase K to each required Lysis Tube

Second, add 10 µL of enrichment to the Lysis Tube

Incubate SureTect Lysis Tubes in the SimpliAmp Thermal Cycler at 36-38°C for 10 minutes followed by 94-96°C for 5 minutes, and 2 minutes at 9-11°C

Transfer 20 µL to SureTect PCR Tubes

Load samples into the QuantStudio 5 and start PCR, review results at end of run (approx. 45 min)

Day 1 (SureTect Automation Workflow)

Prepare the Sample Enrichment Tubes.

Transfer between 700 µL to 1 mL of the enriched sample to each Sample Enrichment Tube

Prepare the Lysis tubes according to instructions. When prompted by the SureTect Automation Software, remove the caps of the Proteinase K tubes and place the reagents in the correct deck position according to the layout. Then when prompted place the Sample Enrichment Tubes with the tube rack into the instrument

Prepare the PCR tubes according to instructions

After the lysis step is complete, the SureTect Automation Software will prompt to load the PCR tubes for the PCR sample preparation step. At this point, remove the seals

Load into the QuantStudio 5 Food Safety PCR Instrument and start PCR, review results at end of run (approx. 50 min)

Report PCR-negative results and confirm PCR-positive results by culturing onto *Brilliance* Salmonella Agar

Day 2

Confirm colonies grown on the *Brilliance* Salmonella Agar using biochemical and serological procedures described in ISO 6579-1:2017 or with an ISO 16140 6:2019 validated confirmatory test

Workflow overview for SureTect Salmonella species, Typhimurium and Enteritidis Multiplex Flex PCR Assay according to ISO 16140 2:2016 standard

Day 0

Primary production samples (PPS): Add 25 g sample to 225 mL Tetrathionate (Hajna) Broth (TT Broth) supplemented with Iodine-iodide

Incubate for 16-20 hours at 36-38°C

Day 1

1 mL of enriched sample is transferred in 9 mL BPW

Incubate for 4-6 hours at 36-38°C

KingFisher Flex Instrument

500 µL of secondary enrichment is loaded onto the KingFisher Flex Instrument

Allow lysates to cool at room temperature for at least 2 min, then transfer 20 µL to SureTect Salmonella PCR Tubes

QuantStudio 5 Food Safety Instrument

Load PCR Tubes into the QuantStudio 5 Food Safety Instrument and then start PCR, review results at end of run (approx. 50 min)

Report PCR-negative results and confirm PCR-positive results by culturing onto *Brilliance* Salmonella Agar

Day 2

Confirm colonies grown on the *Brilliance* Salmonella Agar using Oxoid Salmonella Latex Test (*Salmonella* species), or biochemical and serological procedures (*Salmonella* Enteritidis and *Salmonella* Typhimurium) procedures described in ISO 6579-1:2017 standard or with an ISO 16140 6:2019 validated confirmatory test

Product order information for SureTect Salmonella Multiplex PCR Assay workflows

Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water – (ISO-Meat Peptone)		500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	PrecisBlue Supplement		1 vial for 80x25 g samples	SR0259A
	TT Broth		500 g, makes 10.9 L	CM0671B
	Brilliance™ Salmonella Agar	*Brilliance*™ Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Brilliance Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
	Tryptone Soya Agar		500 g, makes 12.5 L	CM0131B
Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex Assay – for use with the SimpliAmp Thermal Cycler (includes Proteinase K, Lysis Tubes (pre-filled with Lysis Reagent 1), Salmonella multiplex PCR Tubes (pre-filled with Salmonella multiplex PCR tablets), 96-Well Pierceable Seals and PCR Tube caps)			96 tests	A33227
Thermo Scientific™ SureTect™ Salmonella species, Typhimurium and Enteritidis Multiplex Flex Assay – for use with KingFisher Flex Purification System (includes Dynabeads™ anti-Salmonella, Proteinase K, Lysis Reagent 1, Salmonella multiplex PCR Tubes (pre-filled with Salmonella multiplex PCR tablets) and PCR Tube caps)			96 tests	A33227KF
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A
Thermo Scientific™ Remel™ Agglutinating Sera	Salmonella O Factor 4 (Group B)		2 mL vial	R30956901
	Salmonella H (i)		2 mL vial	R30161601
	Salmonella H (1,2)		2 mL vial	R30163301
	Salmonella O Factor 9 (Group D)		2 mL vial	R30957301
Applied Biosystems™ SimpliAmp™ Thermal Cycler (Lysis step)			Instrument	A24811
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (includes Thermo Scientific™ RapidFinder Analysis Software v3.0 or higher and laptop computer)			Instrument	A36328
Thermo Scientific™ SureTect™ Automation Platform, one of the following:		Automation Platform CHOICE Head	Instrument	A59017
		Automation Platform Head R 96	Instrument	A66295
Assurance Service Plan – additional 1-year warranty			Service agreement	SCQS5FSAT

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available. Please contact your local representative or technical services to find out more.

Alternative Method:

Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit workflow

The SureCount Salmonella Multiplex PCR Kit workflow offers a simple, all-in-one solution for *Salmonella* quantification and differentiation of *Salmonella* species, as well as two of the most prevalent serotypes found in meat and poultry matrices: *Salmonella* Typhimurium, and *Salmonella* Enteritidis.

- Quantify three *Salmonella* targets within a single PCR reaction.
- Simple sample preparation provides scalability.
- RapidFinder Analysis Software v3.0 or later provides a quantitative result for all three targets.

Enrichment
Single enrichment step for meat and production environment samples

Lysis
Single enrichment step for meat and production environment samples

Run and read
Results automatically interpreted

Report/confirm
Report PCR-negative results and confirm *Salmonella* spp., SE/ST results with single plate and serology

Validation

The SureCount Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit has been AOAC *Performance Tested Methods*℠ certified. According to the AOAC-PTM validation study, the method is able to quantify 1 CFU per tested poultry rinses, raw meat, and poultry samples (chicken parts and carcass rinses, ground beef, ground pork, and ground turkey.

AOAC RI Certificate No. 072302. Available at **https://assets.thermofisher.com/TFS-Assets/MBD/brochures/AOAC-Cert-SureCount-Salmonella-072302-EN.pdf**

Workflow overview for the SureCount Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit

Day 0

Raw ground turkey

Add 325 g to 1,625 mL of Buffered Peptone Water (BPW) (ISO)

Mix and transfer 30 mL of liquid portion to 30 mL of pre-warmed BPW (ISO) with 12 mg/L Novobiocin or PrecisBlue Supplement

Incubate 42 ± 1°C for 7 h ±10 min

Raw ground beef or pork

Add 325 g to 975 mL of Tryptone Soya Broth (TSB)

Mix and transfer 30 mL of liquid portion to 30 mL of pre-warmed TSB

Incubate 42 ± 1°C for 7 h ±10 min

Chicken parts rinse (finished product) or 30 mL chicken carcass rinse (finished product)

Add 30 mL of rinse to 30 mL of pre-warmed BPW (ISO) with 12 mg/L Novobiocin

Incubate 42 ± 1°C for 7 h ±10 min

Day 1

Remove ~1.5 mL of enrichment to a sterile sealable tube

First, add 10 µL of Proteinase K to each required Lysis Tube

Second, add 10 µL of enrichment to the Lysis Tube

Incubate SureTect Lysis Tubes in the SimpliAmp Thermal Cycler at 36-38°C for 10 minutes followed by 94-96°C for 5 minutes, and 2 minutes at 9-11°C

Transfer 20 µL to SureTect PCR Tubes

QuantStudio 5 PCR Instrument

Load into the QuantStudio 5 Food Safety PCR Instrument and start PCR then review results at end of run (approx. 50 min)

45 min

Report PCR-negative results and confirm PCR-positive results by culturing onto *Brilliance* Salmonella Agar

Day 2

Confirm colonies grown on the *Brilliance* Salmonella Agar using biochemical and serological procedures described in ISO 6579-1:2017 or with an ISO 16140 6:2019 validated confirmatory test

Product order information for SureTect SureCount Salmonella species, Typhimurium and Enteritidis PCR Kit workflow

Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water – (ISO-Meat Peptone)		500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	PrecisBlue Supplement		1 vial for 80x25 g samples	SR0259A
	TSB Broth		500 g, makes 15.2 L	CM0989B
	Brilliance™ Salmonella Agar	*Brilliance*™ Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assay (includes Proteinase K, Lysis Tubes (pre-filled with Lysis Reagent 1), Salmonella multiplex PCR Tubes (pre-filled with Salmonella multiplex PCR tablets), Lysis Tube caps and PCR Tube caps)			96 tests	A56848
Applied Biosystems™ SimpliAmp™ Thermal Cycler (Lysis step)			Instrument	A24811
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (includes Thermo Scientific™ RapidFinder Analysis Software v3.0 or higher and laptop computer)			Instrument	A36328
Assurance Service Plan – additional 1-year warranty			Service agreement	SCQS5FSAT

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Alternative Method:

Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay workflow

A rapid method for the enrichment, detection, and confirmation of *Salmonella* Infantis in poultry carcass rinses and up to 375 g poultry parts samples.

- Designed specifically for *Salmonella* Infantis control programs in poultry production
- The SureTect Salmonella Infantis PCR Assay has been extensively tested and verified on poultry matrices
- Combine the SureTect Salmonella species Typhimurium and Enteritidis Multiplex PCR Kit with the SureTect Salmonella Infantis PCR Assay to simultaneously detect *Salmonella* spp. and identify the top 3 serotypes in poultry products linked to human illness: *S.* Typhimurium, *S.* Enteritidis, and *S.* Infantis.

Enrichment

Single enrichment step for meat and production environment samples

Lysis

Direct lysis and DNA extraction carried out in SimpliAmp Thermal Cycler

Run and read

Results automatically interpreted

Report/confirm

Report PCR-negative results and confirm results with single plate and serology

Validation

The SureTect Salmonella Infantis PCR Assay has been validated internally against the FSIS MLG Method Chapter 4.

Workflow overview for the SureTect Salmonella Infantis PCR Assay

Day 0

Raw poultry parts

375g + 1,125 mL BPW supplemented with 12 mg/L Novobiocin or PrecisBlue Supplement

41.5 ± 1°C for 16–24 hours

1 poultry carcass

Rinse in 400 mL room temperature BPW supplemented with 12 mg/L Novobiocin or PrecisBlue Supplement

41.5 ± 1°C for 16–20 hours

Day 1

Remove ~1.5 mL of enrichment to a sterile sealable tube

First, add 10 µL of Proteinase K to each required Lysis Tube

Second, add 10 µL of enrichment to the Lysis Tube

Incubate SureTect Lysis Tubes in the SimpliAmp Thermal Cycler at 36-38°C for 10 minutes followed by 94-96°C for 5 minutes, and 2 minutes at 9-11°C

Transfer 20 µL to SureTect Salmonella Infantis PCR Tube

QuantStudio 5 PCR Instrument

Load into the QuantStudio 5 Food Safety PCR Instrument and start PCR, then review results at end of run (approx. 50 min)

Report PCR-negative results and confirm PCR-positive results by culturing onto *Brilliance* Salmonella Agar

Day 2

Confirm colonies grown on the *Brilliance* Salmonella Agar using biochemical and serological procedures described in ISO 6579-1:2017 or with an ISO 16140 6:2019 validated confirmatory test

Product order information for SureTect Salmonella Infantis PCR Assay workflows

Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water – (ISO-Meat Peptone)		500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	PrecisBlue Supplement		1 vial for 80x25 g samples	SR0259A
	Salmonella Agar	*Brilliance*™ Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
	Tryptone Soya Agar		500 g, makes 12.5 L	CM0131B
Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay			96 tests/kit	A56450
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A
Thermo Scientific™ Remel™ Agglutinating Sera	Salmonella H (1,5)		2 mL vial	R30163401
	Salmonella H (b,d,E,r)		2 mL vial	R30161201
Applied Biosystems™ SimpliAmp™ Thermal Cycler (Lysis step)			Instrument	A24811
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (includes Thermo Scientific™ RapidFinder Analysis Software v3.0 or higher and laptop computer)			Instrument	A36328

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available. Please contact your local representative or technical services to find out more.

Alternative Method:

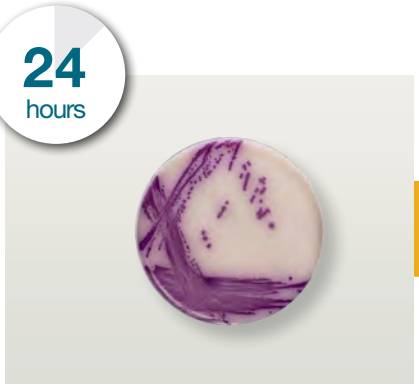
Thermo Scientific™ Salmonella Precis™ Method

A quick and easy method for the enrichment, detection and confirmation of *Salmonella* species from food, animal feed primary production samples and environmental samples.

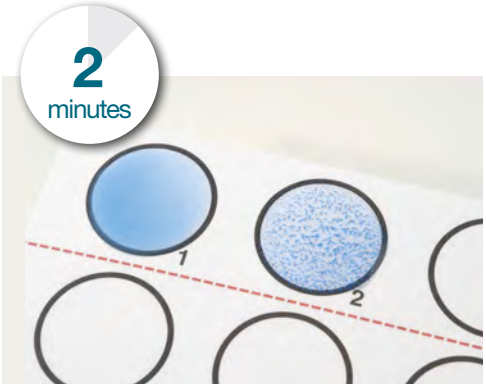
- Validated according to ISO 16140 2:2016 standard against ISO 6579-1:2017
- Simple procedure—no specialized equipment required
- Visible selective enrichment of *Salmonella* testing samples thanks to the inert blue dye of the PrecisBlue Supplement.
- Single overnight enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Quick and convenient confirmation: Salmonella Latex Test or ISO 6579:2017 standard tests
- Reduced time to result: 2 days compared with up to 5 days for standard culture methods
- *Brilliance* Salmonella Agar contains novel Inhibigen™ technology, giving targeted specificity and reduced background flora

Single overnight enrichment

*refer to IFU for exact incubation times and temperatures by sample type



Single 24-hour plate incubation



Rapid and easy confirmation with Salmonella Latex Test or ISO 6579-1 confirmation steps

Reactions on *Brilliance* Salmonella Agar

	Colony color/appearance		
	Purple	Blue	Colorless
Enzyme targeted by chromogen	*Salmonella* (including Lactose positive *Salmonella*)	*Kiebsiella*, *Enterobacter*, *Serratia*	*Citrobactor*, other bacteria and yeasts
Esterase	+	-/+	-
β-glucosidase	-	+	-

E. coli and other bacteria and yeasts are inhibited by the combination of the Inhibigen and other selective agents in the medium.

Validation

The Salmonella Precis method has been validated and approved by the AOAC Research Institute (AOAC-RI) and by NF VALIDATION for AFNOR Certification according to ISO 16140-2:2016 standard against the reference method ISO 6579-1:2017 Detection of *Salmonella* spp.

For flexibility, confirmation was validated using both the Oxoid Salmonella Latex Test and the tests outlined in ISO 6579:2017.

AFNOR Certification validation certificate No. UNI 03/06-12/07 is available in PDF format from the AFNOR website **https://nf-validation.afnor.org/en/food-industry/salmonella-spp/**.

Workflow overview for Salmonella Precis Method validated according to NF VALIDATION for AFNOR Certification

Day 0

Food and feed:

25 g + 225 mL
BPW (ISO)
+ 12 mg/L
Novobiocin
or PrecisBlue
Supplement
20-26 h at 34-38°C
or
25 g + 225 mL
ONE Broth
Salmonella
16-22 h at
40.5-42.5°C

Environmental samples:

- 25 g + 225 mL
- 1 wipe + 225 mL
- 1 swab + 10 mL
- 1 sponge + 100 mL

BPW (ISO)
20-26 h at 34-38°C
or
ONE Broth
Salmonella
16-22 h at
40.5-42.5°C

Up to 375 g Milk powder, infant formula* and infant cereals*:

Up to 375 g
diluted 1:10 with
BPW (ISO)
+ 6 mg/L
Vancomycin
18-24 h at 34-38°C

Up to 375 g Cocoa and chocolate products:

Up to 375 g
diluted 1:10 with
prewarmed
BPW (ISO)
22-28 h at 34-38°C

Recommendations of
the ISO
6887-4:2017 method
20-26 h at 34-38°C

Up to 150 g Animal feed:

Up to 150 g
diluted 1:10 with
BPW (ISO)
+ 12 mg/L
Novobiocin
or PrecisBlue
Supplement
20-26 h at 34-38°C

Day 1

Inoculate onto *Brilliance* Salmonella Agar

Incubate for 22-26 h at 36-38°C

Day 2

Confirm typical colonies as *Salmonella* using Oxoid *Salmonella* Latex Test or Microbact GNB 24E Kit

or

ISO 6579-1:2017 confirmatory tests or with an ISO 16140 6:2019 validated confirmatory test

*with or without probiotics

Product order information for Salmonella Precis Method

Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water	Buffered Peptone Water (ISO)	500 g, makes 25 L	CM1049B
		Buffered Peptone Water (ISO-meat peptone)	500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	PrecisBlue Supplement		1 vial for 80 x 25 g	SR0259A
	Vancomycin Supplement	Vancomycin Supplement - freeze-dried	10 vials of 3 mg	SR0186E
		Vancomycin Supplement - freeze-dried	10 vials of 5 mg	SR0247E
	ONE Broth Salmonella	ONE Broth Salmonella Base	500 g, makes 20 L	CM1091B
		ONE Broth Salmonella Supplement	10 vials, each for 225 mL	SR0242B
	Brilliance™ Salmonella Agar	*Brilliance*™ Salmonella Agar Base	500 g, makes 9.3 L	CM1092B
		Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A
Thermo Scientific™ Oxoid™ Microbact GNB 24E Kit			40 tests/kit	MB1131A

Please note that a range of alternative formats of culture media such as Bagged Enrichment Media and Prepared Plate Media are available.
Please contact your local representative or technical services to find out more.

Learn more at **thermofisher.com/salmonella-testing-food**