

# Food Microbiology Testing Methods: *Salmonella* species

# 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 chemoorganotrophic, 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 refrigerator 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 Centers for Disease Control and Prevention.

## Infective dose

The ingestion of 1-10 cells can constitute a human infectious dose. Low 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, shrimp, salad dressing, cake mixes and chocolate.

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 illustrated 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. Alternative method: Thermo Scientific™ SureTect™ *Salmonella* species PCR Assay workflow
3. Alternative method: Thermo Scientific™ RapidFinder™ *Salmonella* species, Typhimurium and Enteritidis Multiplex PCR Assay methods
4. Alternative method: Thermo Scientific™ *Salmonella* PreciS™ method

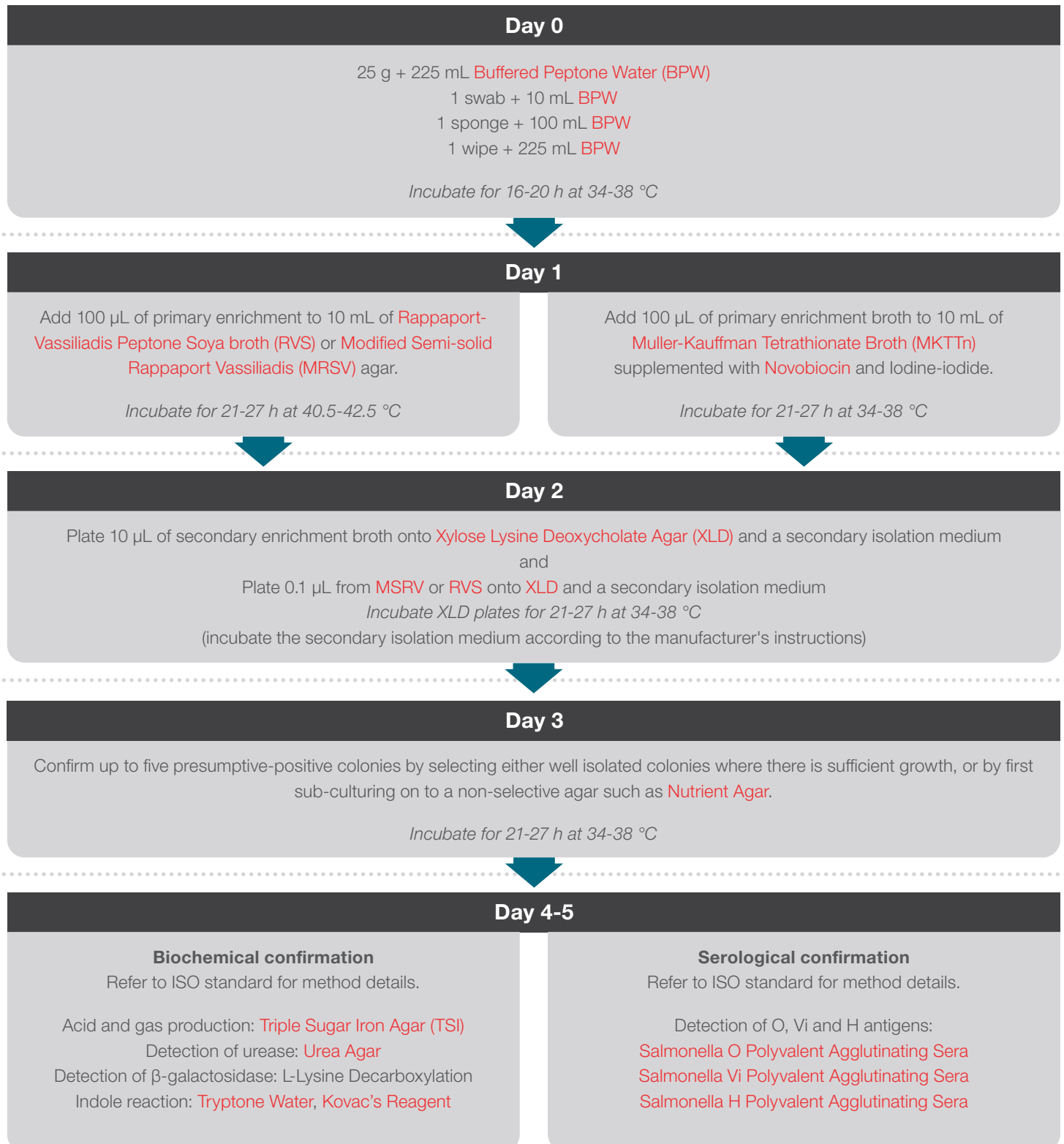
## References

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>:  
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 the ISO 6579 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



**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
		Novobiocin Supplement - freeze-dried	10 vials of 10 mg
	Novobiocin	Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg
	MKTTn Broth	MKTTn Broth Base	500 g, makes 6.1 L
	XLD Agar		500 g, makes 9.4 L
		<i>Brilliance</i> ™ Salmonella Agar Base	500 g, makes 9.3 L
	<i>Brilliance</i> ™ Salmonella Agar	<i>Brilliance</i> ™ Salmonella Agar Selective Supplement	10 vials, each for 500 mL
	Nutrient Agar		500 g, makes 17.8 L
	TSI Agar		500 g, makes 7.7 L
		Urea Agar Base	500 g, makes 20.8 L
	Urea Agar	40% Urea Solution	10 vials, each for 100 mL
	Tryptone Water		500 g, makes 33.3 L
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.

## 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 ISO 16140-part 2:2016 standard
- Streamlined and rapid workflows – 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 the ISO reference method

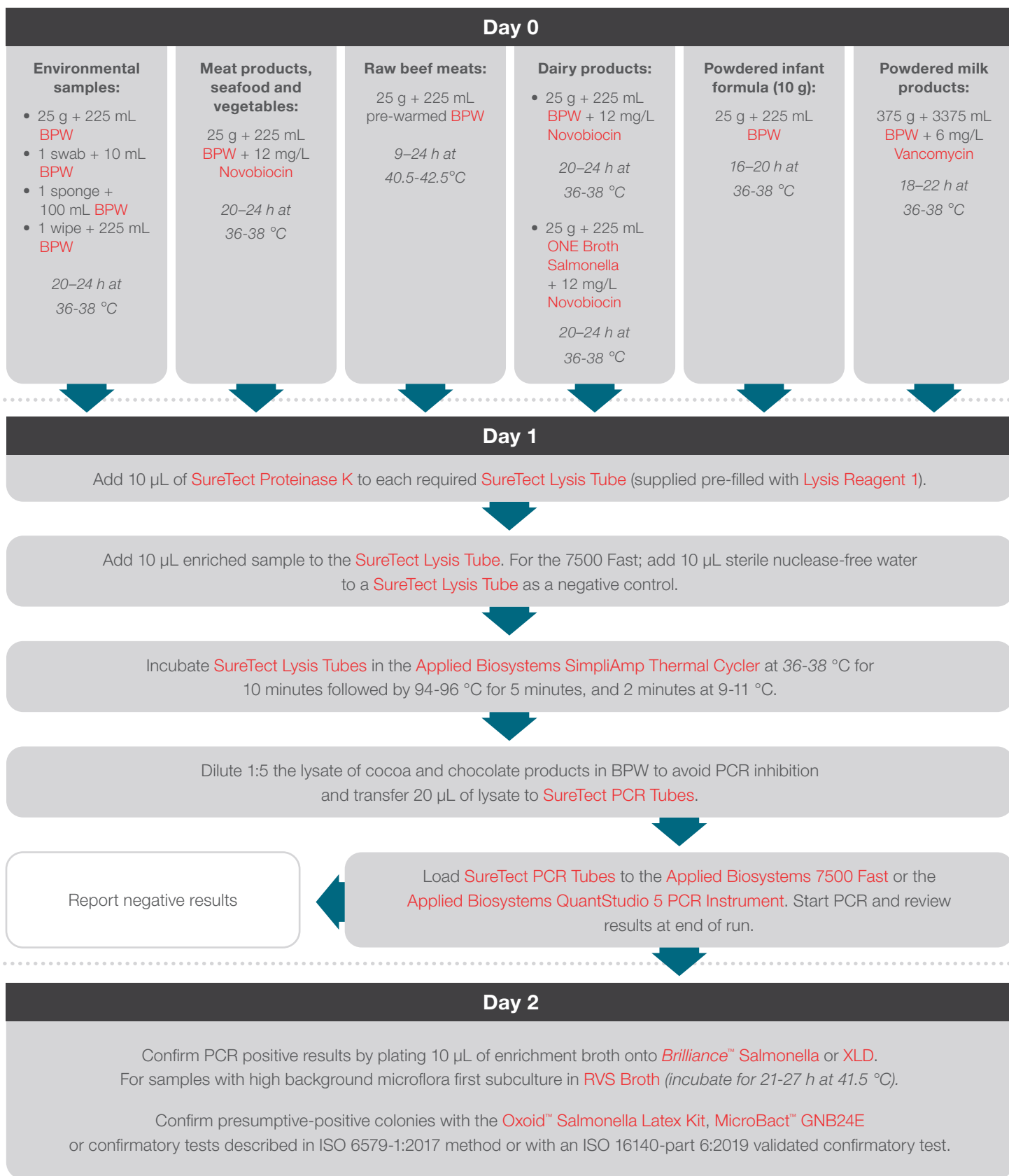


## Validation

The Thermo Scientific™ SureTect™ Salmonella Assay workflow has been validated and approved 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.

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





## Product order information for SureTect Salmonella Species PCR Assay workflow

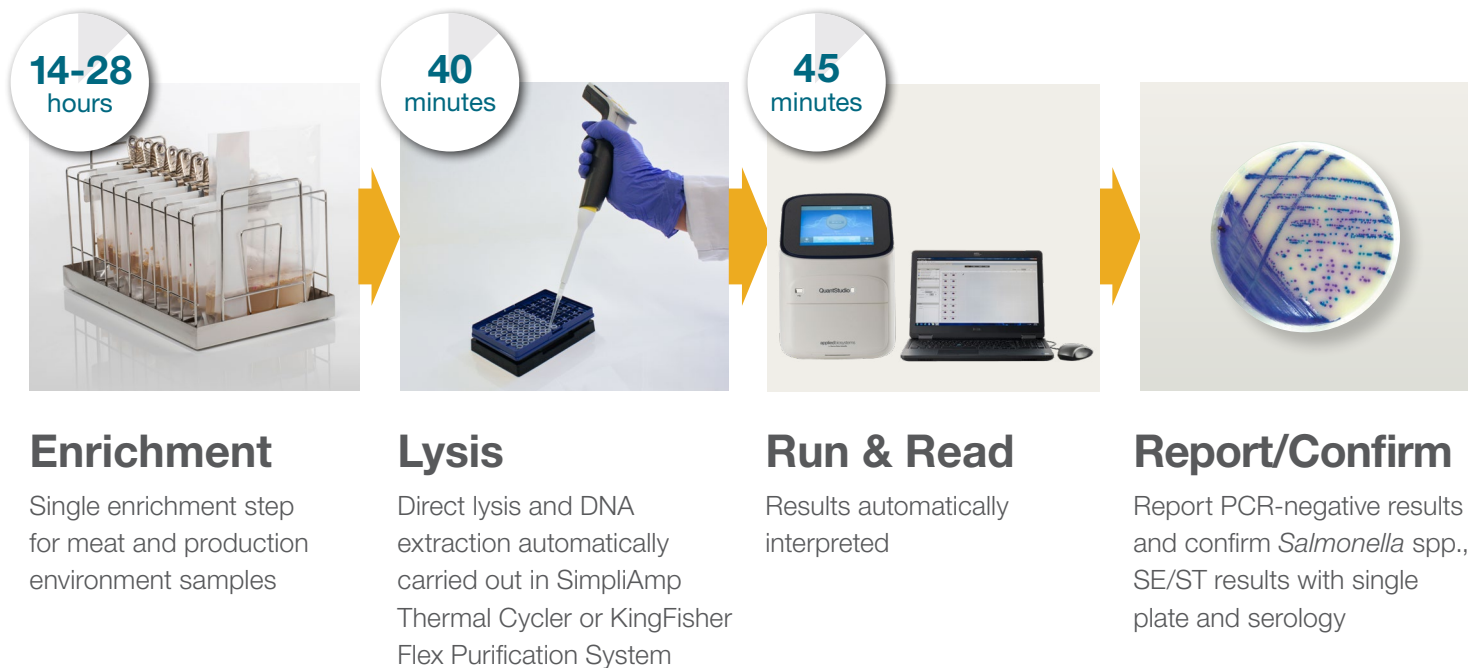
Product description			Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water	Buffered Peptone Water (ISO)	500 g, makes 25 L	CM1049B
		Buffered Peptone Water (ISO-meat peptone)	500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 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	SR0242B
	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
	XLD Agar		500 g, makes 9.4 L	CM0469B
	RVS Broth		500 g, makes 18.7 L	C0866B
Thermo Scientific SureTect Salmonella species Assay (includes Proteinase K, Lysis Reagent 1, Lysis Tubes (pre-filled with Lysis Reagent 1), Salmonella spp. PCR Tubes (pre-filled with Salmonella PCR tablets), Lysis Tube caps and PCR Tube caps)			96 tests	PT0100A
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A
Thermo Scientific™ Microbact GNB24E			80 tests	MB1074A
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 v1.0 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 RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Assays

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, production environment samples and primary production samples (PPS):

- Validated according to ISO 16140-part 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
- Streamlines testing workflow and reduces 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



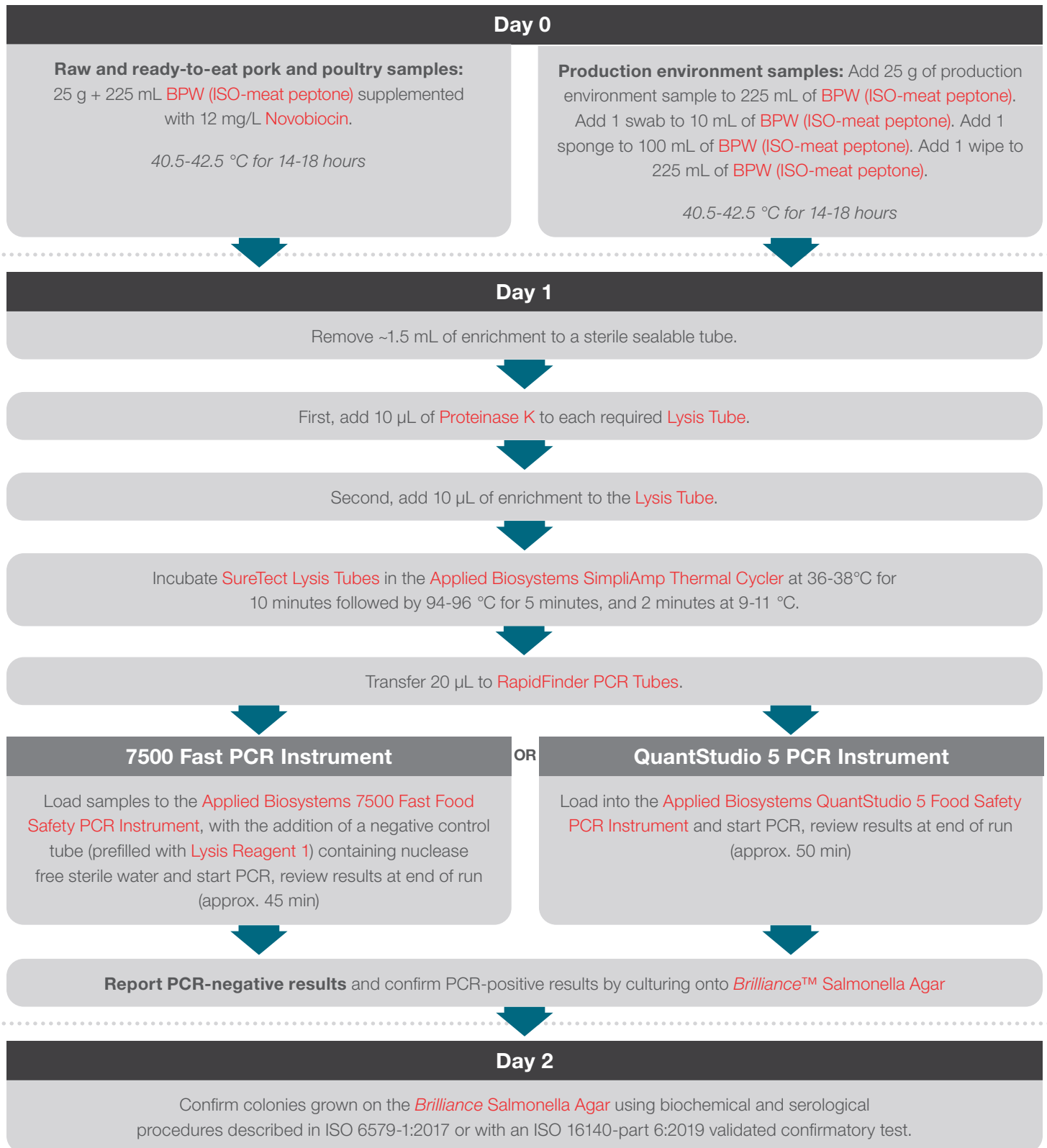
## Validation

The RapidFinder™ Salmonella Multiplex Assay and RapidFinder™ Salmonella Multiplex Flex Assay methods have been validated and approved by NF VALIDATION for AFNOR Certification according to ISO 16140-2:2016 standard against the reference method ISO 6579-1 2017 Detection and serotyping of *Salmonella* spp.

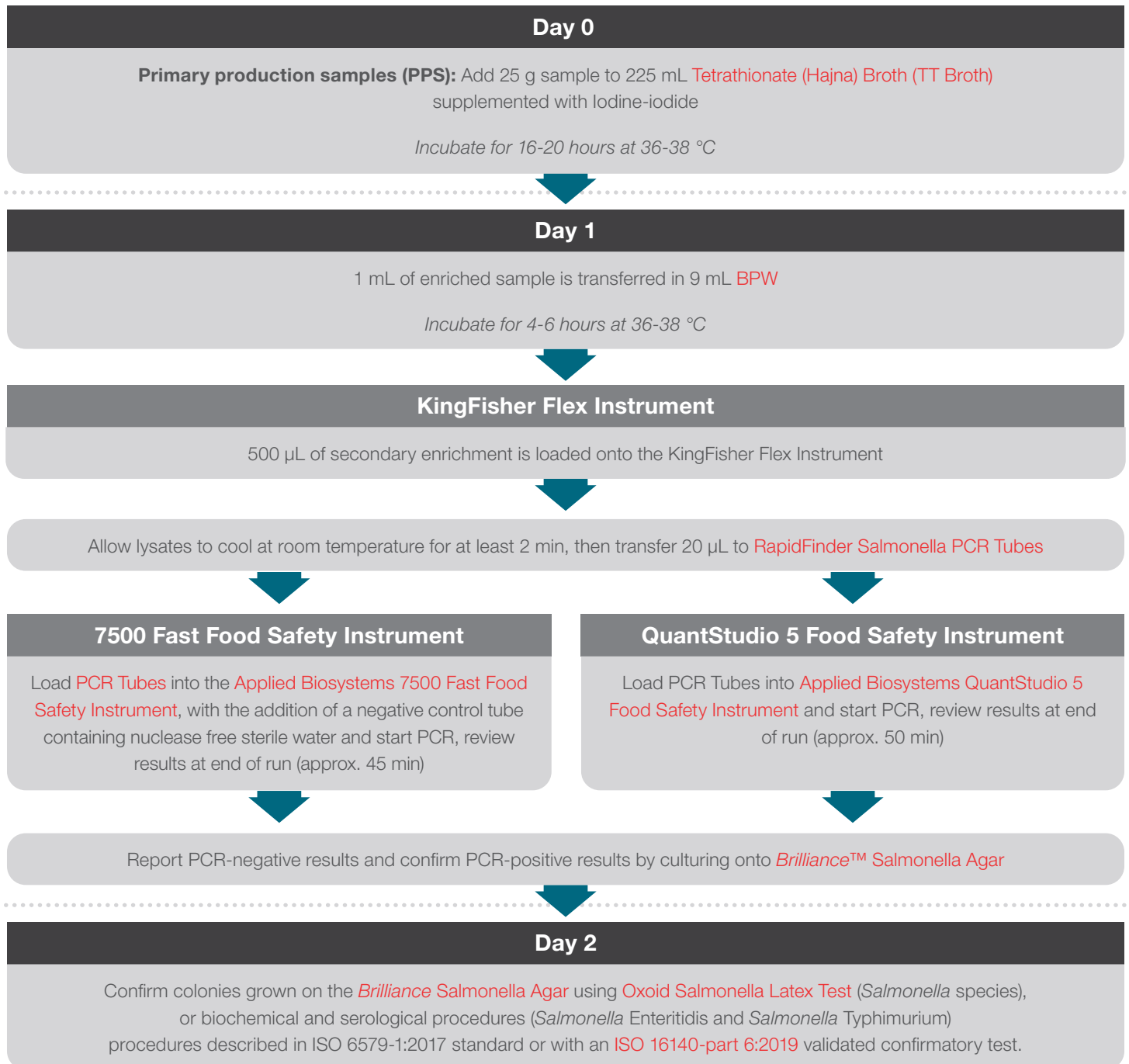
NF VALIDATION certificate No. UNI 03/12-01/18 is available in PDF format from the AFNOR website <https://nf-validation.afnor.org/en/food-industry/salmonella-spp/>.



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



**Workflow overview for Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex Flex PCR Assay according to ISO 16140-part 2:2016 standard**



## Product order information for RapidFinder Salmonella Multiplex PCR Kit workflows

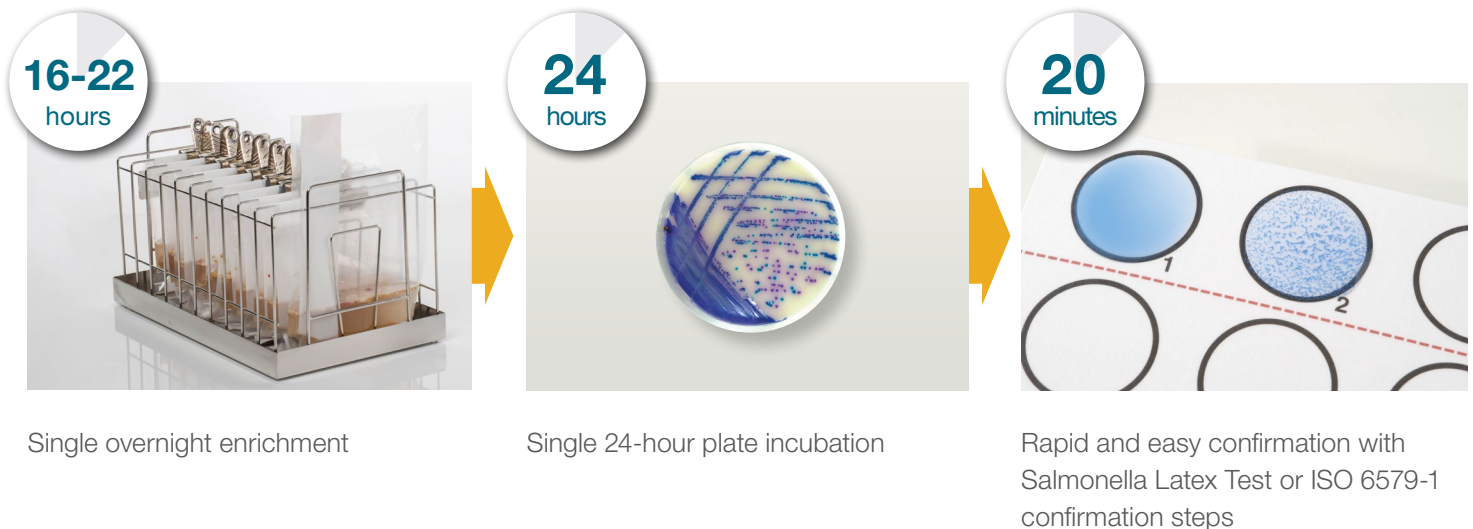
Product description		Format	Order code	
Thermo Scientific™ Oxoid™ Culture Media	Buffered Peptone Water – (ISO-Meat Peptone)	500 g, makes 25 L	CM1211B	
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	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 RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex Assay – for use with the SimpliAmp Thermal Cyclers (includes Proteinase K, Lysis Reagent 1, 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	A33227
	Thermo Scientific RapidFinder 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™ Microbact GNB24E		80 tests	MB1074A	
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	
	Salmonella H (g,m)	2 mL vial	R30161201	
	Salmonella H (1,7)	2 mL vial	R30163601	
Applied Biosystems™ SimpliAmp™ Thermal Cyclers (Lysis step)		Instrument	A24811	
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (includes Thermo Scientific™ RapidFinder Analysis Software v1.0 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 Salmonella Precis Method

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

- Validated according to ISO 16140-part 2:2016 standard against ISO 6579-1:2017
- Simple procedure—no specialised equipment required
- Single overnight enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Quick and convenient confirmation: Oxoid 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



## Reactions on Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar

	Colony colour/appearance		
	Purple	Blue	Colourless
Enzyme targeted by chromogen	<i>Salmonella</i> (including Lactose positive <i>Samonella</i> )	<i>Kiebsiella</i> , <i>Enterobacter</i> , <i>Serratia</i>	<i>Citrobactor</i> , 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.

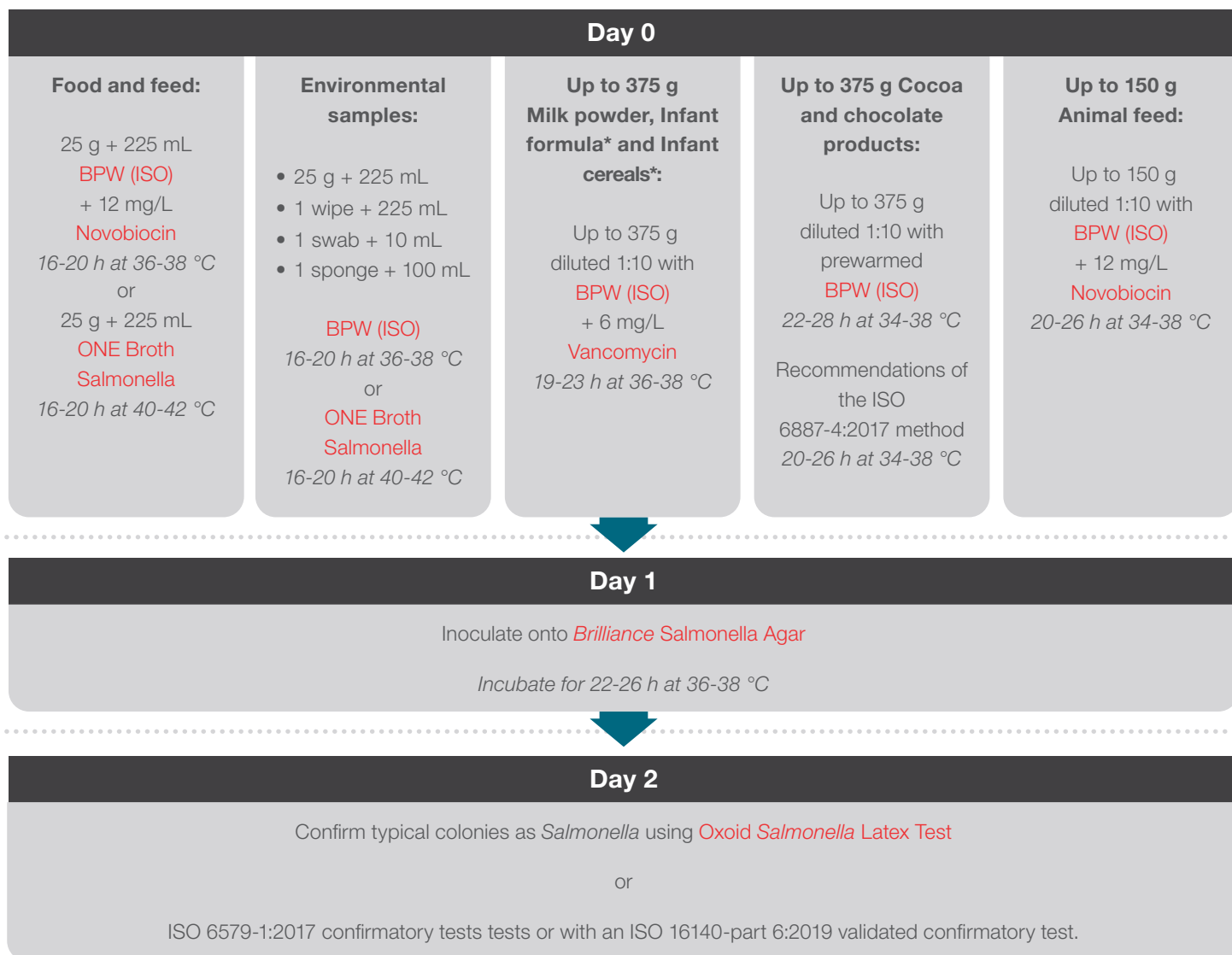
## Validation

The Salmonella Precis™ method has been validated and approved by 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. Alternatively, biochemical panels such as Thermo Scientific™ Microbact™ GNB 24E or Thermo Scientific™ RapID ONE™ Panel, may be used.

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 Thermo Scientific Salmonella Precis Method validated according to NF VALIDATION for AFNOR Certification



\*with or without probiotics



## Product order information for Thermo Scientific 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
	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
		Brilliance™ Salmonella Agar Selective Supplement	10 vials, each for 500 mL	SR0194E
Thermo Scientific™ Oxoid™ Salmonella Latex Kit			100 tests	DR1108A

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.

Find out more at

**[thermofisher.com/salmonella-testing-food](https://thermofisher.com/salmonella-testing-food)**