



Food safety testing

Food Microbiology Testing Methods: *Listeria* species

Listeria species

The genus *Listeria* belongs to the family *Listeriaceae*. *Listeria* are Gram-positive, non-spore forming rods. There are currently 20 recognized species of *Listeria*¹, with *Listeria monocytogenes* being the most clinically significant.

Unlike many other foodborne pathogens, *Listeria* can both survive and grow at refrigeration temperatures. This ability, along with the risk of cross-contamination to other foods and surfaces, contributes to its persistence in food processing environments and makes it a significant concern for food safety.

Incidence

Listeriosis is a foodborne illness commonly caused by *Listeria monocytogenes*. It is a relatively rare but serious infection and can lead to severe symptoms, especially among vulnerable populations such as pregnant women, newborns, the elderly, and individuals with weakened immune systems. Consequently, although not a leading cause of foodborne illness, *Listeria monocytogenes* is among the leading causes of death from foodborne illness.

Infective dose

The infective dose of *Listeria monocytogenes* is undetermined but is believed to vary with the strain and susceptibility of the host. The food source involved may also impact the dose-response relationship. In cases associated with raw or inadequately pasteurized milk, for example, it is likely that fewer than 1,000 cells may cause disease in susceptible individuals².

Foods affected

Many foods have been associated with *Listeria monocytogenes*. Examples include raw milk, inadequately pasteurized milk, chocolate milk, cheeses (particularly soft cheeses), ice cream, raw vegetables, raw poultry and meats (all types), fermented raw-meat sausages, hot dogs and deli meats, and raw and smoked fish and other seafood. As mentioned previously, *Listeria monocytogenes* can grow in refrigerated temperatures, which makes this organism a particular problem for the food industry².

Potential contamination sources include food worker exposure, airborne transmission, raw materials, and food processing environments. Among these, post-processing contamination on food-contact surfaces poses the greatest threat to product contamination².

References

1. Encyclopedia of Dairy Sciences, 2022, 3rd edition
2. FDA – The Bad Bug Book – Version 2 - 2012

Methods overview

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

Detection

1. Reference method: ISO 11290-1:2017 (en)
Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method. This method is cited in Commission Regulation (EC) No 2073/2005 and other national regulations and guidelines.
2. Reference method: FDA BAM Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples. Cited in the U.S Food & Drug Administration Bacteriological Analytical Manual.
3. Reference method: USDA FSIS MLG 8.15: Isolation and identification of *Listeria monocytogenes*: A culture-media based standard reference method cited in the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Method Number 8.15.
4. Proprietary method, PCR: Thermo Scientific™ SureTect™ *Listeria* species PCR Assay Workflow.
5. Proprietary method, PCR: Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay Workflow.
6. Proprietary method, Chromogenic media: Thermo Scientific™ *Listeria* PreciS Detection Methods.

Enumeration:

1. Reference method: ISO 11290-2:2017(en)
Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method
2. Reference method: FDA BAM Chapter 10: Enumeration of *Listeria monocytogenes* in Foods.
3. Proprietary method, Chromogenic media: Thermo Scientific™ *Listeria* PreciS Enumeration Methods.

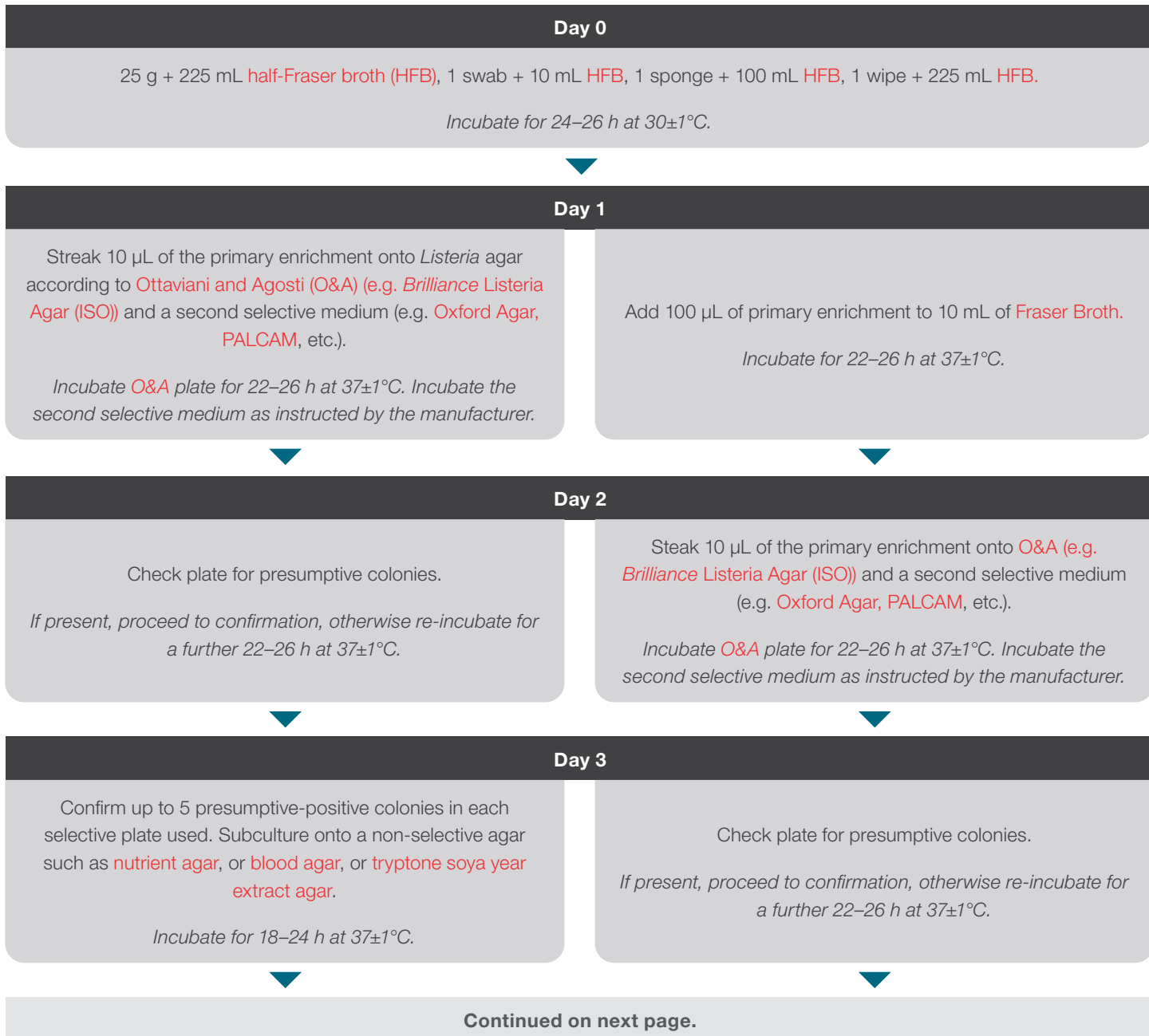
Detection

1. Reference method: ISO 11290-1:2017 (en)

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1

The ISO 11290:2017 standard outlines a method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* species in food, animal feed, and environmental samples. Below is an outline of the workflow steps for Part 1.

Part 1 (Detection) workflow overview



Day 4

See confirmations.

Confirm up to 5 presumptive-positive colonies in each selective plate used. Subculture onto a non-selective agar such as **nutrient agar**, **blood agar**, or **Tryptone Soya Agar with Yeast Extract**.

Incubate for 18–24 h at 37±1°C.

Confirmations

For the full list of confirmations and instructions, refer to the reference method directly.

Listeria monocytogenes

Mandatory tests:

Microscopy
β-haemolysis
L-rhamnose
D-xylose

Optional tests:

Catalase
Mobility at 25°C
CAMP

Listeria species

Mandatory tests:

Microscopy
Catalase

Optional tests:

Voges-Proskauer
Mobility at 25°C

Product order information to help you implement this method:

Reference method medium	Product description	Format	Order code
Thermo Scientific™ Oxoid™ and Remel™ Culture Media	Fraser Broth Base (Dehydrated)	500 g/ Makes 8.7 L medium	CM0895B
	Fraser Supplement	10 vials	SR0156E
	Half Fraser Broth	10 bottles	BO0350S
	Half Fraser Broth Ready Bags	3 x 3 L bags	FB1381B
	<i>Brilliance</i> Listeria Agar (ISO) Base (Dehydrated)	500 g	CM1212B
	<i>Brilliance</i> Listeria Agar (ISO) Selective Supplement	1 vial / 500 mL agar	SR0257E
	<i>Brilliance</i> Listeria Agar (ISO) Differential Supplement	1 vial / 500 mL agar	SR0258E
	PALCAM Agar Base (Dehydrated)	500 g	CM0877B
	PALCAM Selective Supplement	10 vials	SR0150E
	Listeria Selective Oxford Agar Base (Dehydrated)	500 g/ Makes 9 mL medium	CM0856B
	Listeria Selective Supplement	10 vials	SR0140E
	Remel Oxford Modified Agar Base (Dehydrated)	500 g	R454232
	Oxford Listeria Selective Supplement	10 vials	SR0206E
	Nutrient Agar Base (Dehydrated)	500 g	CM0003B
	Tryptone Soya Agar with Yeast Extract	10 x 90 mm plates	PO5050A
	Tryptone Soya Agar with Sheep Blood	10 x 90 mm plates	PB5012A
	Blood Agar Base (Dehydrated)	500 g/ Makes 12.5 L of medium	CM0055B
	Horse Blood Supplement	100 mL	SR0035C

From bags to bottles: our culture media comes in a range of alternative formats, giving you flexibility and convenience in designing your workflow. [Get in touch with your local Thermo Fisher Scientific microbiology representative to find out more](#)

Detection

2. Reference method: FDA BAM Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples.

The FDA BAM Chapter 10 outlines the method for the detection of *Listeria monocytogenes*.

Workflow overview

Day 0

25 g + 225 mL **Buffered Listeria Enrichment Broth (BLEB)**,
1 swab or sponge (pre-moistened in 10 mL Dey/Engley (D/E) neutralising broth) + 90 mL **BLEB**

Incubate for 4 hour at 30±1°C.

Then aseptically add filter sterilized selective agents to the **BLEB** to achieve the final concentrations of:

10 mg/L acriflavine
50 mg/L cycloheximide
40 mg/L sodium nalidixic acid

Mix well and continue to incubate for a total of 24–48 h at 30±1°C.

Day 2

Refer to BAM Chapter 10 section F for alternate screening methodologies.

If negative after 48 h incubation, report negative result. If positive:

Streak 10 µL of the **BLEB** enrichments onto one esculin-based (e.g. **Oxford agar**, **PALCAM**, etc.)
and one chromogenic selective agar (e.g. **O&A (Brilliance Listeria Agar (ISO))**).

Incubate the plates at the specified temperatures for 24–48 h.

Day 4

Check plate for presumptive colonies. If no colonies are present after 48 h, report as negative. If presumptive colonies are present:

Confirm up to 5 presumptive-positive colonies in each selective plate used.

Subculture onto **Tryptone Soya Agar Yeast Extract (TSAYE)**.

Incubate for 24–48 h at 30°C.

Confirmations

Select well-isolated colonies to choose from standard classification and alternative rapid identification.

For the full list of confirmations and instructions, refer to the reference method directly.

Standard classification:

Haemolysis
CAMP test
Mobility
Catalase
Gram stain
Carbohydrate fermentation series

Alternative rapid identification:

API Listeria
MICRO-ID Listeria Identification System
VITEK 2 Automatic Gram Positive card
Real-Time PCR

Product order information to help you implement this method:

Reference method medium		Product description	Format	Order code
Thermo Scientific™ Oxoid™ and Remel™ Culture Media	Buffered Listeria Enrichment Broth (BLEB)	Buffered Listeria Enrichment Broth Base (Dehydrated)	500 g	CM0897B
	Esculin agar options	PALCAM Agar Base (Dehydrated)	500 g	CM0877B
		PALCAM Selective Supplement	10 vials	SR0150E
		Listeria Selective Oxford Agar Base (Dehydrated)	500 g/ Makes 9 mL medium	CM0856B
		Listeria Selective Supplement	10 vials	SR0140E
		Remel™ Oxford Modified Agar Base (Dehydrated)	500 g	R454232
		Oxford Listeria Selective Supplement	10 vials	SR0206E
	Chromogenic agars	<i>Brilliance</i> Listeria Agar (ISO) Base (Dehydrated)	500 g	CM1212B
		<i>Brilliance</i> Listeria Agar (ISO) Selective Supplement	1 vial / 500 mL agar	SR0257E
		<i>Brilliance</i> Listeria Agar (ISO) Differential Supplement	1 vial / 500 mL agar	SR0258E
	TSAYE	Tryptone Soya Agar with Yeast Extract	10/Pk	R110690

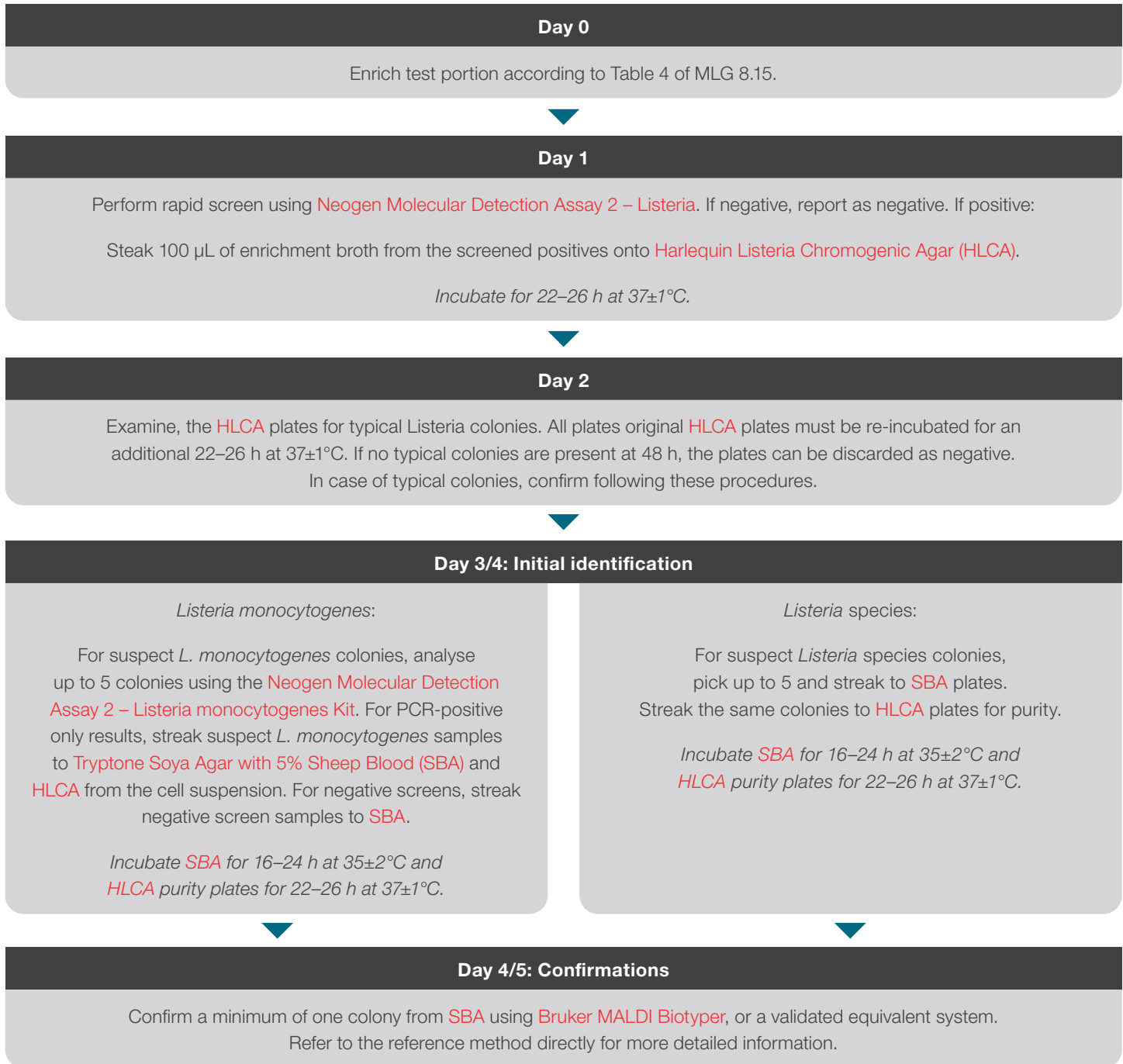
From bags to bottles: our culture media comes in a range of alternative formats, giving you flexibility and convenience in designing your workflow. [Get in touch with your local Thermo Fisher Scientific microbiology representative to find out more](#)

Detection

3. Reference method: USDA FSIS MLG 8.15: Isolation and identification of *Listeria monocytogenes*

The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Method Number 8.14 outlines the method for the isolation and identification of *Listeria monocytogenes*.

Workflow overview



Detection

4. Proprietary Method: Thermo Scientific™ SureTect™ *Listeria* species PCR Assay Workflow

Detect *Listeria* species simply, reliably, and rapidly in even the most challenging food types with the SureTect *Listeria* Species PCR Assay, which incorporates optimized primer and probe components for highly-specific detection of *Listeria* species.

- Easy-to-use with single enrichment step and pre-dispensed reagents
- Target-specific colored assay plastics
- Clearly oriented lysis and assay trays
- Color indicator in lysis reagent reducing possibility for human error
- Option to automate the lysis and PCR sample preparation
- Optimized workflow delivers on-screen results within a day
- Reduced time-to-result: 1 day compared with up to 5 days for reference methods

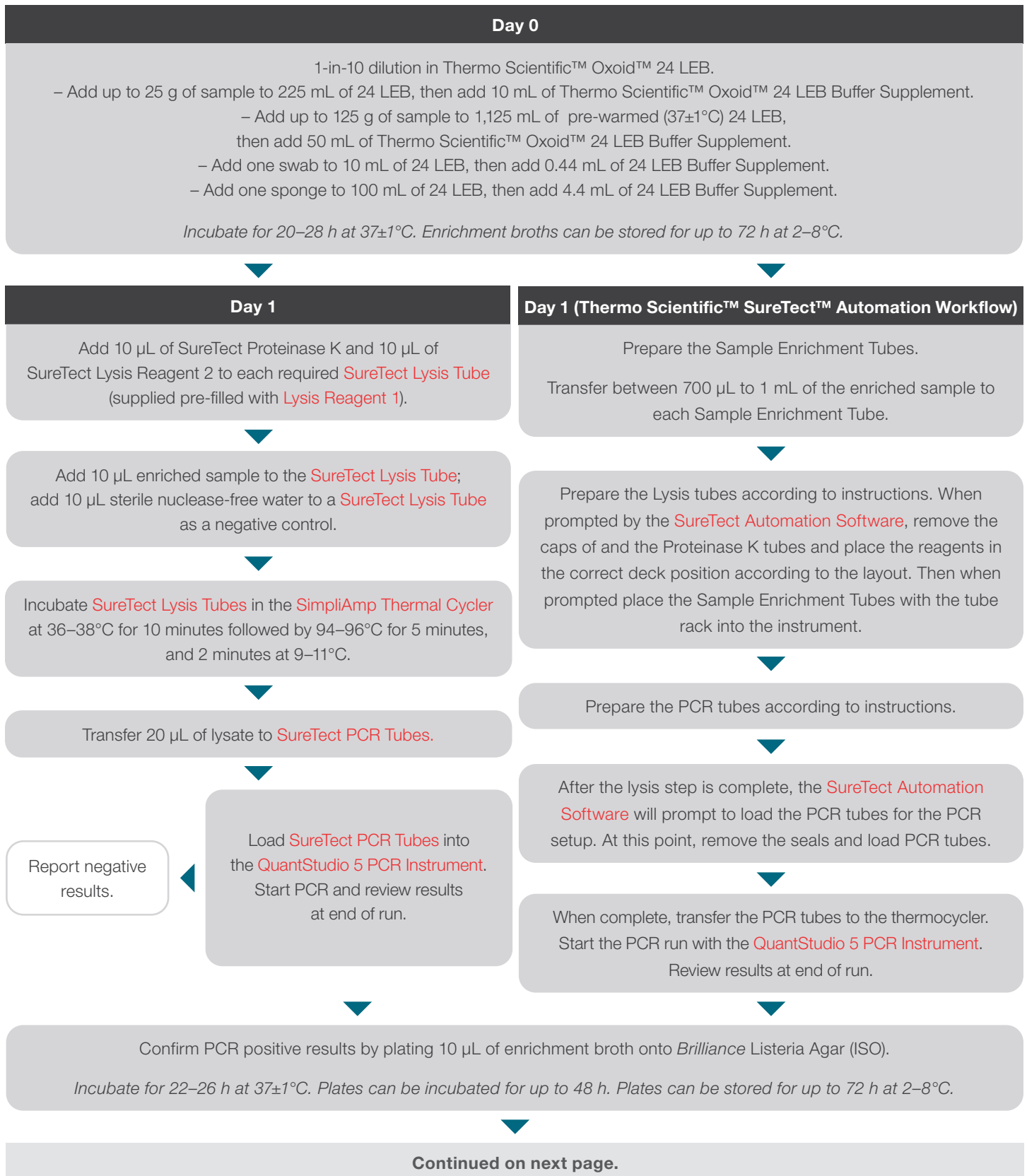


Validation

The assay's workflow holds AOAC *Performance Tested Methods*SM ([AOAC-PTM & OMA](#)) approval granted by the AOAC Research Institute and is an AOAC *Official Method*SM (2021.06), with Final Action status granted by AOAC International. The assay is also validated according to ISO 16140-2:2016 and certified by NF ValidationTM ([AFNOR \(ISO 16140-2\)](#)) by AFNOR Certification.

Workflow overview for the SureTest Listeria species PCR Assay according to ISO 16140 2:2016 standard

Workflow overview



Day 2

For NF Validation, the presence of characteristic colonies on the *Brilliance* Listeria Agar (ISO) is enough to confirm the positive result.

Product order information for the SureTect Listeria species PCR Assay workflow

Product description		Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	24 Listeria Enrichment Broth (24 LEB) (Dehydrated)*	500 g	CM1107B
	24 Listeria Enrichment Broth Complete (24 LEB Complete)	500 g	CM1154B
	24 Listeria Enrichment Broth (24 LEB) Selective Supplement	10 pk/ 5 mL per bottle	SR0243E
	24 Listeria Enrichment Broth (24 LEB) Buffer Supplement	10 pk/ 100 mL per bottle	BO1204M
	<i>Brilliance</i> Listeria Agar Base (ISO)	500 g	CM1212B
	<i>Brilliance</i> Listeria Agar (ISO) Selective Supplement	1 vial per 500 mL agar	SR0257E
	<i>Brilliance</i> Listeria Agar (ISO) Differential Supplement	1 vial per 500 mL agar	SR0258E
SureTect Listeria species PCR Assay		96 tests/ 1 kit	A56842
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
SureTect Automation Platform, one of the following:	Automation Platform CHOICE Head	Instrument	A59017
	Automation Platform Head R 96	Instrument	A66295

*(requires the addition of 2 vials of SR0243E)

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Detection

5. Proprietary Method: Thermo Scientific™ SureTest™ *Listeria monocytogenes* PCR Assay Workflow

Detect *Listeria monocytogenes* in even the most challenging food types with the SureTest *Listeria monocytogenes* PCR Assay, which incorporates optimized primer and probe components for highly-specific detection of *L. monocytogenes*.

- Easy-to-use with single enrichment step and pre-dispensed reagents
- Target-specific colored assay plastics
- Clearly oriented lysis and assay trays
- Color indicator in lysis reagent reducing possibility for human error
- Option to automate the lysis and PCR sample preparation
- Optimized workflow delivers on-screen results within a day



Validation

The assay's workflow holds AOAC Performance Tested MethodsSM ([AOAC-PTM & OMA](#)) approval granted by the AOAC Research Institute and is an AOAC Official MethodSM (2021.05), with First Action status granted by AOAC International. The assay is also validated according to ISO 16140-2:2016 and certified by NF ValidationTM (AFNOR [\(ISO 16140-2\)](#) by AFNOR Certification. The assay is certified to test a broad range of foods and environmental samples.

Workflow overview for the SureTect *Listeria monocytogenes* PCR Assay according to ISO 16140-2:2016 standard

[See page 8 for the harmonized SureTect workflow.](#)

Product order information for the SureTect *Listeria monocytogenes* PCR Assay workflow

Product description		Format	Order code
Thermo Scientific™ Oxoid™ Culture Media	24 <i>Listeria</i> Enrichment Broth (24 LEB) (Dehydrated)	500 g	CM1107B
	24 <i>Listeria</i> Enrichment Broth Complete (24 LEB Complete)	500 g	CM1154B
	24 <i>Listeria</i> Enrichment Broth (24 LEB) Selective Supplement	10 pk/ 5 mL per bottle	SR0243E
	24 <i>Listeria</i> Enrichment Broth (24 LEB) Buffer Supplement	10 pk/ 100 mL per bottle	BO1204M
	<i>Brilliance</i> ™ <i>Listeria</i> Agar Base (ISO)	500 g	CM1212B
	<i>Brilliance</i> <i>Listeria</i> Agar (ISO) Selective Supplement	1 vial per 500 mL agar	SR0257E
	<i>Brilliance</i> <i>Listeria</i> Agar (ISO) Differential Supplement	1 vial per 500 mL agar	SR0258E
	Microbact™ <i>Listeria</i> 12L Kit	20 Tests/ 1 kit	MB1128A
SureTect <i>Listeria monocytogenes</i> PCR Assay		96 tests/ 1 kit	A56843
SimpliAmp Thermal Cycler (Lysis step)		Instrument	A24811
QuantStudio 5 Food Safety Real-Time PCR System (includes RapidFinder Analysis Software v3.0 or higher and laptop computer)		Instrument	A36328
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

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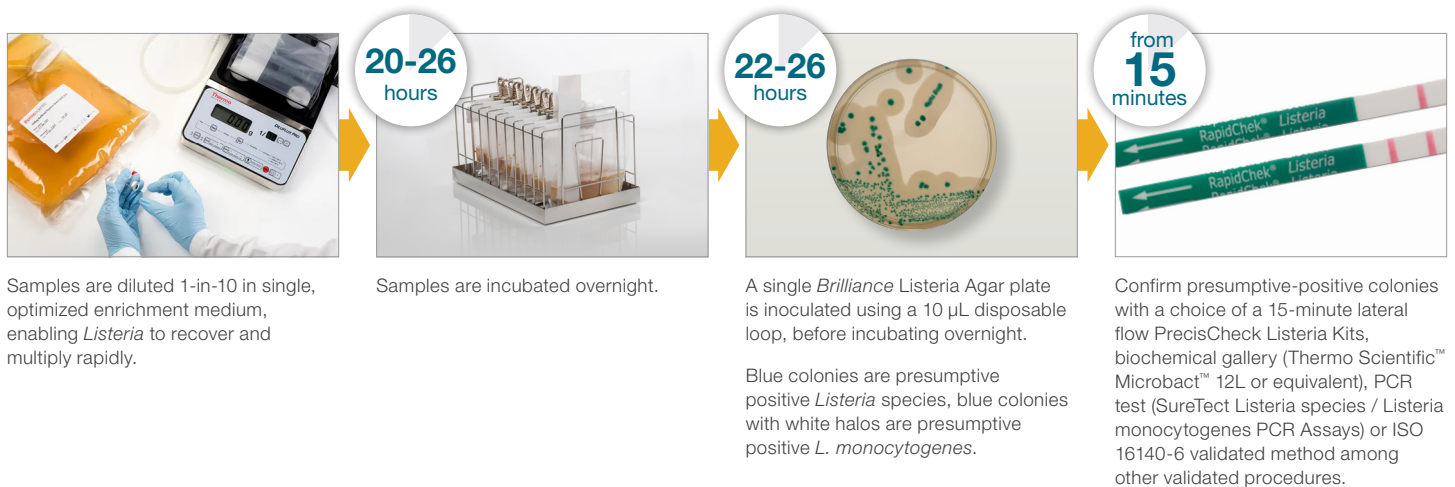
Detection

6. Proprietary Method: Chromogenic media: Thermo Scientific™ Listeria Precis Detection Method

Listeria Precis Methods are rapid culture-based methods for the isolation, enumeration, and identification of *Listeria* spp. and *L. monocytogenes* from food and environmental samples.

- Simple, streamlined procedure—no specialized equipment required.
- Single overnight enrichment.
- Single plate incubation.
- 10 µL loop inoculation of plates, reducing contamination risk and handling steps.
 - 33% reduction in plate inoculation time compared to other, rapid, validated culture-based methods by using a disposable loop, rather than a pipette and tip.
- All testing completed within 48 hours due to a 15-minute rapid confirmation option, Thermo Scientific™ PrecisCheck™ Kits, which can confirm presumptive positive colonies directly from the *Brilliance* Listeria Agar (ISO) plate.

Listeria Precis Detection Method



Listeria Precis Enumeration Method



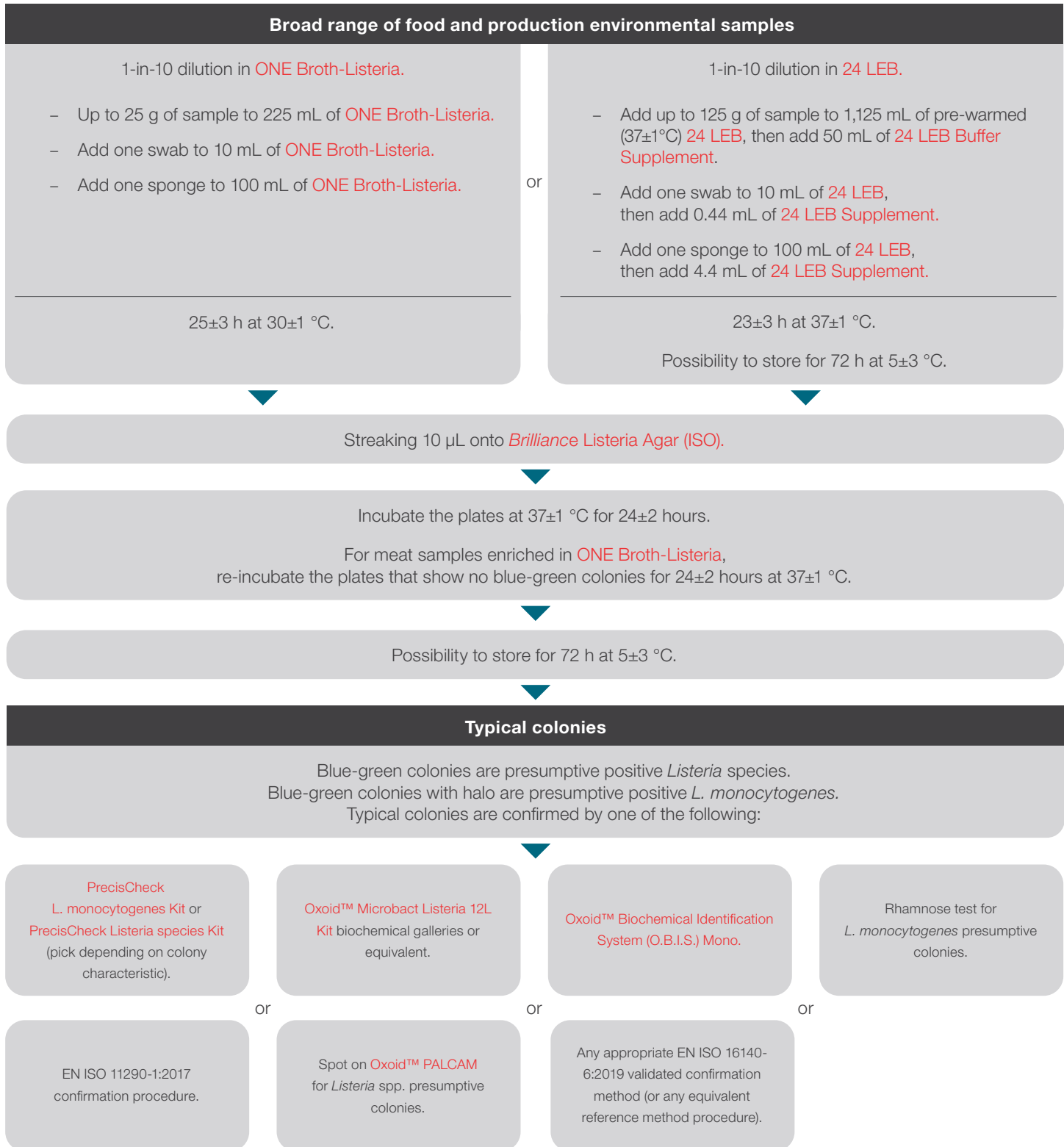
Validation

Listeria Precis Methods are validated for a broad range of samples according to ISO 16140-2 following NF VALIDATION rules when compared to the ISO 11290-1:2017 and ISO 11290-2:2017 Microbiology of the food chain – Horizontal methods for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* species reference methods. The NF VALIDATIONS are detailed below:

- Listeria Precis (Detection *Listeria* species) – UNI 03/14-06/22.
- Listeria Precis (Detection *Listeria* monocytogenes) – UNI-03-04-04-05
- Listeria Precis (Enumeration *Listeria* monocytogenes) – UNI 03/05-09/06
- Listeria Precis (Enumeration *Listeria* species) – UNI 03/15-12/22

Workflow overview for the *Listeria* Precis Detection Method for *Listeria* spp. and *L. monocytogenes*

Workflow overview



Some *L. ivanovii* strains can as well show a typical halo due to a positive lecithinase activity, but these halos are smaller than the halos around *L. monocytogenes* colonies.

Product order information for the Listeria Precis Detection Method for *Listeria* spp. and *L. monocytogenes*

Product description		Format	Order code	
Thermo Scientific™ Oxoid™ Culture Media		24 LEB Broth (dehydrated culture medium)	500 g (makes 7 L)	CM1107B
	24 Listeria Enrichment Broth (24 LEB)	24 LEB Selective Supplement (Listeria Precis Detection Method only)	10 vials (each for 500 mL)	SR0243E
		24 LEB Buffer Supplement (added to all formats of 24 LEB)	24 bottles of 10 mL (each for 225 mL)	BO1339E
		ONE Broth-Listeria	ONE Broth Listeria Base (dehydrated culture medium)	500 g (makes 11.3 L)
			2.5 kg makes 57 L	CM1066R
	ONE Broth Listeria Selective Supplement		10 vials (each for 500 mL)	SR0234E
	ONE Broth Listeria Ready Bag		3 x 3 L bags	FB1380B
	Brilliance Listeria Agar (ISO)	Brilliance Listeria Agar (ISO) Base (dehydrated culture medium)	500 g (makes 7 L)	CM1212B
			5 Kg (makes 70 L)	CM1212T
		Brilliance Listeria Agar (ISO) Bottle		BO1370Z
		Brilliance Listeria Agar (ISO) Selective Supplement	10 vials (each for 500 mL)	SR0257E
			10 vials (each for 200 mL)	SR0257B
		Brilliance Listeria Agar (ISO) Differential Supplement	10 vials (each for 500 mL)	SR0258E
			10 vials (each for 200 mL)	SR0258B
	Microbact Listeria 12L Kit	20 tests	MB1128A	
	PALCAM Medium		PO5104A	
PrecisCheck Listeria species Kit	45 test strips	LF0100A		
PrecisCheck L. monocytogenes Kit	50 test strips	LF0200A		
Biochemical Identification System (O.B.I.S.) Mono	60 tests	ID0600M		
SureTect Listeria species PCR Assay	96 tests	A56842		
SureTect Listeria monocytogenes PCR Assay	96 tests	A56843		

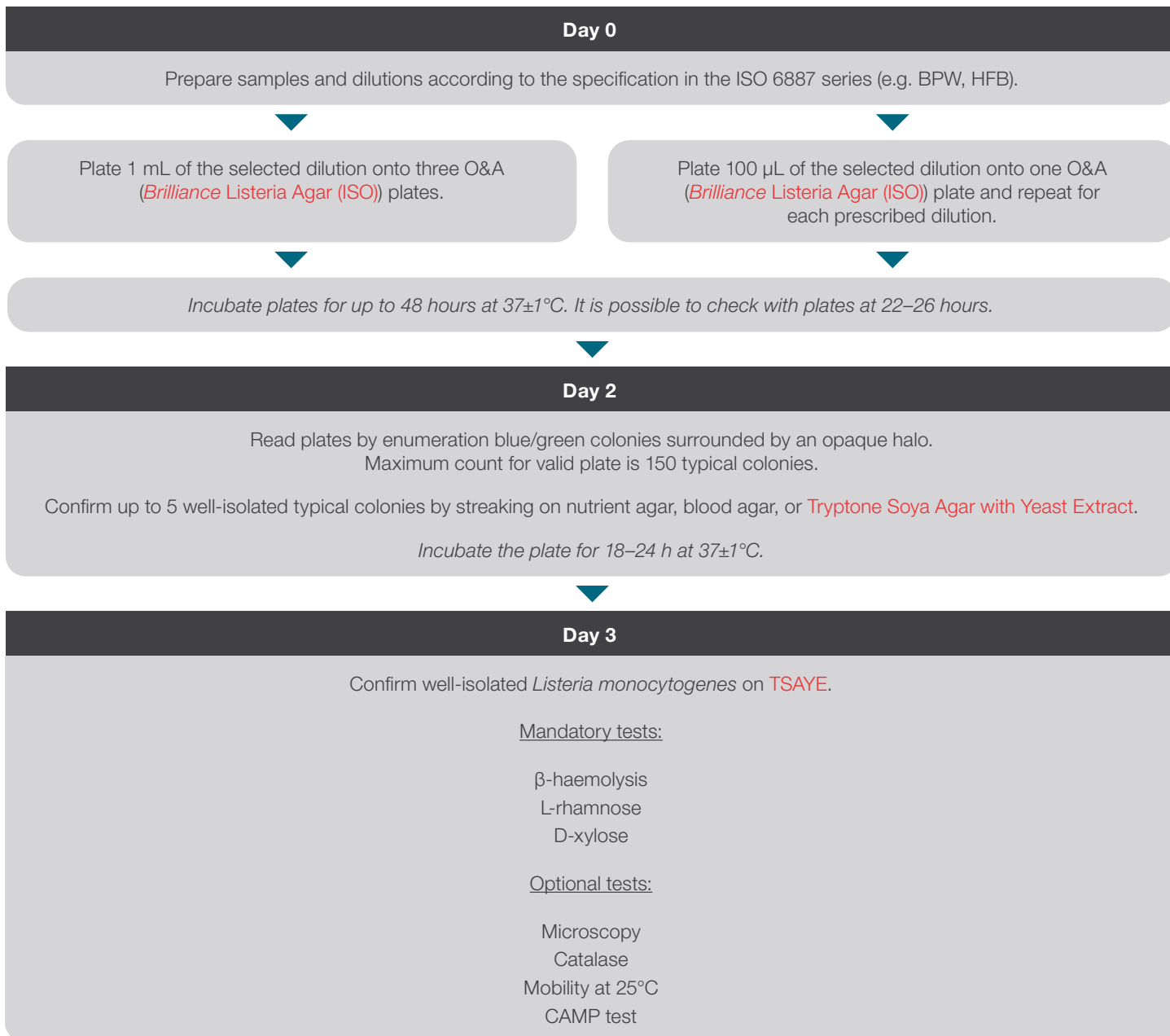
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Enumeration

1. Reference method: ISO 11290-2:2017: Enumeration of *Listeria monocytogenes* and *Listeria* species

The ISO 11290-2:2017 standard outlines a method for the enumeration of *Listeria monocytogenes* and *Listeria* species in food, animal feed, and environmental samples. Below is an outline of the workflow:

Workflow overview



Product order information for testing according to ISO 11290-2:2017: Enumeration of *Listeria monocytogenes* and *Listeria species*

Reference method medium	Product description	Format	Order code	
Thermo Scientific™ Oxoid™ Culture Media	ISO 6887 Diluents	Fraser Broth Base (Dehydrated)	500 g/ Makes 8.7 L medium	CM0895B
		Fraser Supplement	10 vials	SR0156E
		Buffered Peptone Water Base (Dehydrated)	500 g	CM0509B, CM1049B, or CM1211B
	Listeria Agar according to Ottaviani & Agosti (O&A)	<i>Brilliance</i> Listeria Agar (ISO) Base (Dehydrated)	500 g	CM1212B
		<i>Brilliance</i> Listeria Agar (ISO) Selective Supplement	1 vial per 500 mL agar	SR0257E
		<i>Brilliance</i> Listeria Agar (ISO) Differential Supplement	1 vial per 500 mL agar	SR0258E
	Non-selective agar	Nutrient Agar Base (Dehydrated)	500 g	CM0003B
		Tryptone Soya Agar with Yeast Extract	10 x 90 mm plates	PO5050A
		Tryptone Soya Agar with Sheep Blood	10 x 90 mm plates	PB5012A
		Blood Agar Base (Dehydrated)	500 g/ Makes 12.5 L of medium	CM0055B
Horse Blood Supplement		100 mL	SR0035C	

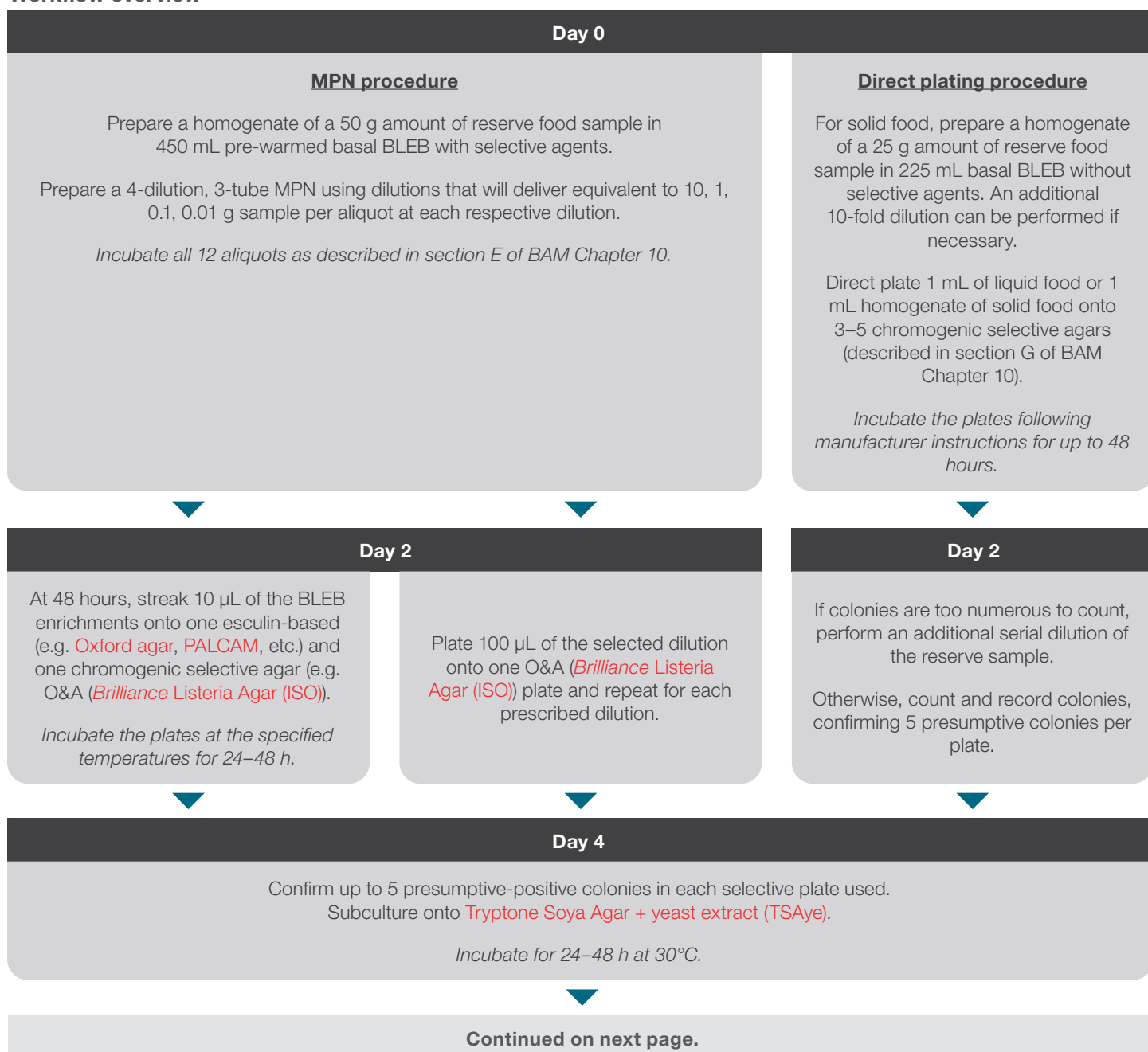
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Enumeration

2. Reference method: FDA BAM Chapter 10: Part 2: Method for enumeration of *Listeria monocytogenes*

The FDA Bacteriological Analytical Manual (BAM) Chapter 10 outlines the method for the detection and enumeration of *Listeria monocytogenes*.

Workflow overview



Day 6 Confirmations

Select well-isolated colonies to choose from standard classification and alternative rapid identification.
For the full list of confirmations and instructions, refer to the reference method directly.

Standard classification:

Haemolysis
CAMP test
Mobility
Catalase
Gram stain
Carbohydrate fermentation series

Alternative rapid identification:

API Listeria
MICRO-ID Listeria Identification System
VITEK 2 Automatic Gram Positive card
Real-Time PCR

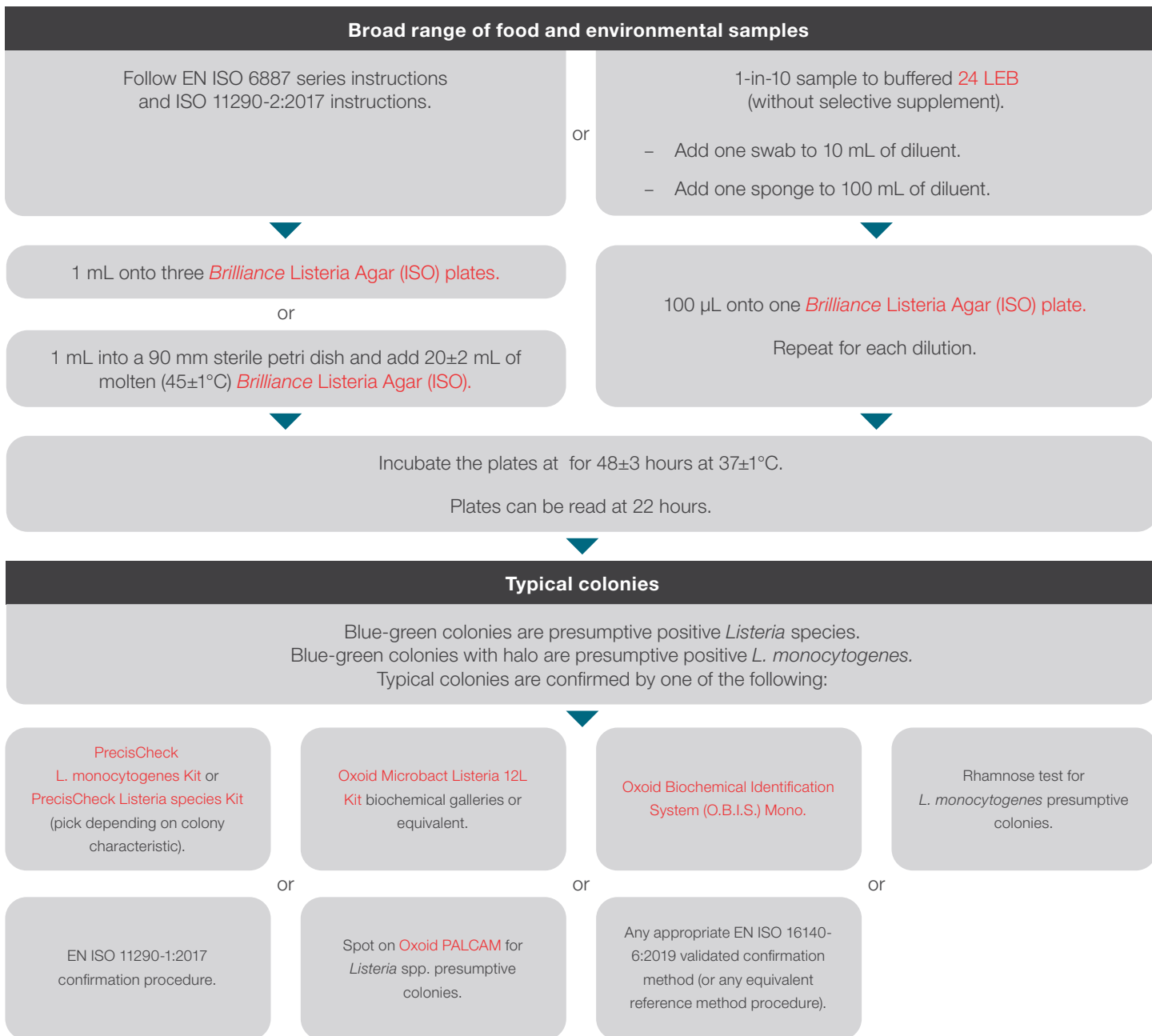
Day 7

Interpret the results following the guidance in BAM Chapter 10 Section J and Appendix 2.

Enumeration

3. Proprietary method: Thermo Scientific Listeria Precis Enumeration Method

Workflow overview for the Listeria Precis Enumeration Method



Product order information for the Listeria Precise Enumeration Method

Reference method medium		Product description	Format	Order code
Thermo Scientific™ Oxoid™ and Remel™ Culture Media	Buffered Listeria Enrichment Broth (BLEB)	Buffered Listeria Enrichment Broth (Dehydrated)	500 g	CM0897B
	Esculin agar options	PALCAM Agar Base (Dehydrated)	500 g	CM0877B
		PALCAM Selective Supplement	10 vials	SR0150E
		Listeria Selective Oxford Agar Base (Dehydrated)	500 g/ Makes 9 mL medium	CM0856B
		Listeria Selective Supplement	10 vials	SR0140E
		Remel Oxford Modified Agar Base (Dehydrated)	500 g	R454232
		Oxford Listeria Selective Supplement	10 vials	SR0206E
	Chromogenic agars	Brilliance Listeria Agar (ISO) Base (Dehydrated)	500 g	CM1212B
		Brilliance Listeria Agar (ISO) Selective Supplement	1 vial per 500 mL agar	SR0257E
		Brilliance Listeria Agar (ISO) Differential Supplement	1 vial per 500 mL agar	SR0258E
	TSAYE	Tryptic Soy Agar w/ Yeast Extract	10/ pk	R110690

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