

HPA STANDARD METHOD

DETECTION AND ENUMERATION OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES

F 19

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections

DETECTION AND ENUMERATION OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES

Issue no: 3.1 Issue date: 13.01.09 Issued by Standards Unit, Department for Evaluations, Standards and Training in conjunction with
the Regional Food, Water and Environmental Coordinators Forum Page 1 of 18
Reference no: F 19i3.1

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Suggested citation for this document:

Health Protection Agency (2009). *Detection and Enumeration of Listeria monocytogenes and other Listeria species*. National Standard Method F 19 Issue 3.1
http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

DETECTION AND ENUMERATION OF LISTERIA MONOCYTOGENES AND OTHER LISTERIA SPECIES

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AMENDMENT PROCEDURE

Controlled document reference	F 19
Controlled document title	Detection and Enumeration of <i>Listeria monocytogenes</i> and other <i>Listeria</i> species

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment		
7/ 13.01.09	3	3.1	6	Scope	Information giving the lower limit of enumeration added		
			6	Background	The sentence regarding the volume for enumeration was expanded to include 0.5mL per 90mm plate.		
			7	Principle	Use of API identification system added		
			7	Safety considerations	Subculture to two selective agar plates added along with incubation temperature		
			7	Safety considerations	Safety information regarding use of ZYM B reagent added		
			8	Culture media	Chromogenic media CLA is replaced with <i>Listeria</i> chromogenic agar with a rider added to indicate other chromogenic media can be used if shown to be equivalent.		
							ALOA and OCLA formulation added
							Oxford agar is added as a second selective agar plate for the detection method.
			10	6.1.1	Added: 'Food intended for infants less than 12 months'		
					Added: 'transfer to a container capable of closure'		
			10	6.1.2	Temperature tolerances added		
			10	6.1.3	Incubation of both <i>Listeria</i> chromogenic and Oxford agar added		
			11	6.3.1	Recognising colonies on <i>Listeria</i> chromogenic and Oxford agar added		
11	6.4.1	Subculture of presumptive colonies to horse blood agar added					

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			12	6.4.2	Reference to use of UV light and ZYM B reagent is moved into an information note. Citation of ISO references removed for clarity.
			12	6.4.4	Control for Listeria chromogenic agar added
			14	Reference facilities	Link to referral forms added
				Appendix 1	Updated
				Appendix 2	Comparison of the HPA and ISO methods inserted
			18	References	Updated

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DETECTION AND ENUMERATION OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES

INTRODUCTION

Scope

The method described is applicable to the detection and enumeration of *Listeria monocytogenes* and other *Listeria* species in all food types including milk and dairy products and in environmental swabs.

In general, the lower limit of enumeration of this method is 1 cfu per millilitre of sample for liquid products, or 10 cfu per gram of sample for other products.

Background

European legislation containing microbiological food safety criteria for *L. monocytogenes*² came into force in England on 11.1.06³. These criteria either specify absence in 25 g of sample or a level below 100 colony forming units (cfu) per gram at any point in the shelf life of the ready-to-eat food. *L. monocytogenes* results exceeding the food safety criteria are judged to be legally unsatisfactory. There is also a requirement for producers of ready-to-eat foods that may pose a *L. monocytogenes* risk to public health to sample the food processing areas and equipment for the presence of *L. monocytogenes* as part of their sampling scheme².

Current PHLS/HPA guidelines for some ready-to-eat foods⁴ contain guideline criteria for total *Listeria* species. The presence of species of *Listeria* other than *L. monocytogenes* is used to indicate the likelihood that *L. monocytogenes* may also be present in other parts of the batch of food or environment. Samples containing more than 100 cfu/g of other *Listeria* species are considered unsatisfactory and their presence above this level requires investigation.

In order to assess the level of contamination in these foods direct enumeration of the organism is carried out on solid selective media. In some ready-to-eat foods such as soft ripened cheeses, pâtés and vacuum or modified atmosphere packed cooked meats with a long assigned shelf life, the very presence of *Listeria* is significant due to the organism's ability to multiply to significant levels during refrigerated storage. For these foods, an enrichment procedure is also required to determine presence or absence in a defined quantity of food.

The method described is based on BS EN ISO 11290 parts 1 and 2^{5,6}. These are internationally recognised horizontal methods for the detection and enumeration of *L. monocytogenes*. A *Listeria* chromogenic isolation medium is used that results in the formation of blue-green colonies by *Listeria* species due to the β -glucosidase activity of these bacteria. Further distinction between the species is obtained by the inclusion of phosphatidylinositol or lecithin⁷⁻⁹ which is hydrolysed by the phospholipase enzyme produced by *L. monocytogenes* and *L. ivanovii* but not other *Listeria* species to produce an opaque halo around the colony.

This method differs from the current ISO 11290-1 and 11290-2 (incorporating Amendment No. 1:2004) in a number of minor ways. However both of these ISO methods are currently under review. The differences between the procedures described in F19 and the proposed revised ISO methods include the volume to be applied to the agar for enumeration in F19 is 0.5 mL per 90 mm plate instead of 0.1 mL (or 1 mL across three 140 mm plates) and F19 describes the use of API identification system which is not incorporated in the current standard but is incorporated in the draft ISO 11290. A more detailed list of differences is available in Appendix 2.

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1 PRINCIPLE

In foods or swabs that require presence/absence testing or where low numbers of organisms in foods may be significant, detection of *L. monocytogenes* and other *Listeria* species necessitates a primary enrichment at 30°C for 24 hours in a selective enrichment broth containing half the normal concentration of nalidixic acid and acriflavine. This is followed by secondary enrichment in the same selective enrichment broth containing the full concentration of selective agents with incubation at 37°C for up to 48 hours. Sub-culture to two selective agar media are made from both enrichment stages. The selective agars are examined for the presence of typical colonies and identification of the species by means of morphological and biochemical tests.

The enumeration of *L. monocytogenes* and other *Listeria* species by this method involves inoculation of the surface of a selective agar media with a specified volume of a 10⁻¹ and other appropriate decimal dilutions of the test sample. *Listeria* chromogenic agar plates are incubated at 37°C for up to 48 hours. Calculation of the number of cfu per gram or mL of sample for either *L. monocytogenes* or total *Listeria* species is made from the number of typical colonies obtained on the selective media, and subsequently confirmed by morphological and biochemical tests.

2 DEFINITIONS

For the purpose of this method, the following definitions apply:

Listeria species

Micro-organisms which form typical colonies on solid selective media, and which display the morphological and biochemical characteristics described in this method.

Listeria monocytogenes

Micro-organisms that conform to the above definition for *Listeria* species, usually display β-haemolysis on horse blood agar, produce acid from rhamnose but not xylose, give a negative reaction in the DL-alanine β-naphthylamide (DIM) test after addition of ZYM B reagent using the API *Listeria* test kit.

Detection of L. monocytogenes and other Listeria species

Determination of the presence or absence of these micro-organisms in a defined weight or volume of food or dairy product or in an environmental sample.

Enumeration of L. monocytogenes and other Listeria species

Determination of the number of these micro-organisms per gram or mL of food or dairy product.

3 SAFETY CONSIDERATIONS¹⁰⁻¹²

Normal microbiology laboratory precautions apply. Women known to be pregnant or think they may be should be excluded from working with known cultures of *Listeria monocytogenes*. ZYM B is toxic and may impair fertility and cause harm to the unborn child. Infection caused by *Listeria monocytogenes* in pregnancy is rare but can result in complications including miscarriage and neonatal infection depending on the trimester of infection.

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4 EQUIPMENT

Usual laboratory equipment and in addition:

- Top pan balance capable of weighing to 0.1 g
- Stomacher
- Gravimetric diluter (optional)
- Vortex mixer
- Spiral plater (optional)
- Incubators at 30°C ± 1°C, 37°C ± 1°C
- Colony counter (optional)
- Light microscope: x 40 objective
- Stomacher bags (sterile)
- Automatic pipettors and sterile pipette tips capable of delivering 0.1 mL, 1 mL and 10 mL volumes (optional)
- Pipettes (sterile total delivery) 10 mL and 1 mL graduated in 0.1 mL volumes (optional)
- Spreaders (sterile)
- UV lamp (365 nm wavelength)

5 CULTURE MEDIA

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

Peptone saline diluent (maximum recovery diluent) (optional)

Peptone	1.0 g
Sodium chloride	8.5 g
Water	1 L
pH 7.0 ± 0.2 at 25°C	

Buffered peptone water (optional)

Peptone	10.0 g
Sodium chloride	5.0 g
<i>di</i> -Sodium hydrogen phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g
Water	1 L
pH 7.2 ± 0.2 at 25°C	

Sodium citrate diluent (optional)

<i>tri</i> -Sodium citrate dihydrate (Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O)	20.0 g
Water	1 L
pH 7.5 ± 0.2 at 25°C	

Di-potassium hydrogen phosphate diluent (optional)

<i>di</i> -Potassium hydrogen phosphate (K ₂ HPO ₄)	20.0 g
Water	1 L
pH 7.5 ± 0.2 or 8.4 ± 0.2 at 25°C	

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Half Fraser and Fraser broth

	Half Fraser	Fraser
Proteose peptone	5.0 g	5.0 g
Tryptone	5.0 g	5.0 g
Meat extract	5.0 g	5.0 g
Yeast extract	5.0 g	5.0 g
Sodium chloride	20.0 g	20.0 g
<i>di</i> -Sodium hydrogen phosphate	12.0 g	12.0 g
Potassium dihydrogen phosphate	1.35 g	1.35 g
Aesculin	1.0 g	1.0 g
Lithium chloride	3.0 g	3.0 g
Ferric ammonium citrate	0.5 g	0.5 g
Nalidixic acid	10 mg	20 mg
Acriflavine hydrochloride	12.5 mg	25 mg
Water	1 L	1 L

pH 7.2 ± 0.2 at 25°C

Horse blood agar

Columbia agar with 5 % horse blood

Listeria Chromogenic Agar (OCLA or ALOA)⁷⁻⁹ (ISO Formulation)

Note: Other chromogenic media for *Listeria* isolation may be used if shown to be equivalent however a full validation using ISO 16140 will be necessary if the formulation used differs from that stated in ISO 11290

	ALOA	OCLA
Enzymatic digest of animal tissues	18.0 g	18.0 g
Enzymatic digest of casein	6.0 g	6.0 g
Yeast extract	10.0 g	10.0 g
Sodium pyruvate	2.0 g	2.0 g
Glucose	2.0 g	2.0 g
Magnesium glycerophosphate	1.0 g	1.0 g
Magnesium sulphate (anhydrous)	0.5 g	0.5 g
Sodium chloride	5.0 g	5.0 g
Lithium chloride	10.0 g	10.0 g
<i>di</i> -Sodium hydrogen phosphate (anhydrous)	2.5 g	2.5 g
Soya lecithin (containing at least 30% phosphatidylinositol)	-----	2.0 g
L- α -Phosphatidylinositol	2.0 g	-----
5-Bromo-4-chloro-3-indolyl- β -D-glucopyranoside	0.05 g	0.05 g
Cycloheximide	0.05 g	
or Amphotericin B	0.01 g	0.01 g
Nalidixic acid sodium salt	0.02 g	0.02 g
Ceftazidime	0.02 g	0.02 g
Polymixin B sulphate	76,700 IU	76,700 IU
Agar	12 - 18.0 g	12 - 18.0 g
Water	1 L	1 L

pH 7.2 ± 0.2 at 25°C

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*Listeria selective agar (Oxford agar)*¹³

Columbia blood agar base	39.0 g
Aesculin	1.0 g
Ferric ammonium citrate	0.5 g
Lithium chloride	15.0 g
Cycloheximide	0.4 g
or Amphotericin B	0.01 g
Colistin sulphate	20.0 mg
Acriflavine	5.0 mg
Cefotetan	2.0 mg
Fosfomicin	10.0 mg
Water	1 L
pH 7.0 ± 0.2 at 25°C	

Gram stain reagents (optional)

Commercial biochemical identification system – API Listeria and ZYM B reagent (see section 3 for relevant safety note)

6 SAMPLE PROCESSING

6.1 SAMPLE PREPARATION FOR DETECTION

6.1.1 SAMPLE PREPARATION AND PRE-ENRICHMENT

Enrichment is necessary for presence/absence testing in 25 g samples, environmental swabs and food samples such as pâté, vacuum or modified atmosphere packed products with extended shelf-life and foods intended for infants less than 12 months old.

Using sterile instruments and aseptic technique, weigh a representative 25 g sample of food into a sterile stomacher bag. Using a stomacher, homogenise with nine times that weight or volume of half Fraser broth. Record the weight of sample and the weight or volume of half Fraser broth used. If the amount of food product available is less than 25 g or mL maintain the sample:diluent volume at 1:9 (1 in 10).

For environmental swabs, ensure that the swab is completely immersed in half Fraser broth, such that an approximate 1 in 10 dilution is achieved. Vortex or stomach to bring the organisms into suspension. Transfer the homogenate or swab suspension into a container capable of closure (such as a bag or screw topped container)¹⁴.

6.1.2 INCUBATION AND ENRICHMENT

Place the primary enrichment (half Fraser) broth in an incubator at 30°C ± 1°C for 24 ± 2 hours. Sub-culture 0.1 mL of the incubated half Fraser broth to 10 mL of Fraser broth and place in an incubator at 37°C ± 1°C for 48 ± 2 hours.

6.1.3 SUBCULTURE TO SELECTIVE AGARS

Sub-culture the primary enrichment (half Fraser) broth cultures after 24 ± 2 hours to *Listeria* chromogenic agar and oxford agar. Sub-culture the secondary enrichment (Fraser) broth cultures after 48 ± 2 hours to both *Listeria* chromogenic and oxford agar plates. Incubate *Listeria* chromogenic and oxford agar plates aerobically at 37°C ± 1°C.

Examine the plates for presence of typical colonies as described in section 6.3 after incubation for 24 ± 3 hours and after a further 24 ± 3 hours if necessary.

6.2 SAMPLE PREPARATION FOR ENUMERATION

Following the procedure described in National Standard Method F 2 (foods) or D 1 (dairy products), prepare a 10⁻¹ homogenate in peptone saline diluent (PSD), buffered peptone water (BPW) or other suitable diluent (see D 1). If necessary, also prepare a 10⁻³ dilution in PSD. If

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only *Listeria* is sought the 10⁻¹ homogenate may be prepared in unsupplemented Fraser broth and the half Fraser supplement added after inoculation of the enumeration plates.

Inoculate 0.5 mL of the 10⁻¹ dilution onto the surface of two *Listeria* chromogenic agar plates. Carefully spread the inoculum as soon as possible over the surface of the plates using a sterile spreader. If counts are expected to be high use a spiral plater to inoculate 50 µL of the 10⁻¹ and 10⁻³ dilutions onto *Listeria* chromogenic agar plates.

Leave the plates on the bench for approximately 15 minutes to allow absorption of the inoculum into the agar. Invert the plates and place in an incubator at 37°C ± 1°C for 24 ± 3 hours and a further 24 ± 3 hours if necessary.

6.3 RECOGNITION AND COUNTING OF COLONIES

6.3.1 RECOGNITION

Examine the plates for typical colonies of *Listeria* species after 24 ± 3 hours and again after a further 24 ± 3 hours if necessary.

In an outbreak investigation incubation of agar plates may need to be extended to 96 hours if there is no growth or no typical colonies cultured after 48 hours¹⁵.

Listeria chromogenic agar

Colonies of *Listeria* appear blue or blue-green. Typical colonies of *L. monocytogenes* are surrounded by an opaque halo after 24 hours; this halo may be weak or slow to develop if the organism is stressed, particularly acid-stressed. Strains of *L. ivanovii* also develop an opaque halo, but within 48 hours. Other species of *Listeria* do not develop a halo. Blue colonies may also be formed by other species such as *Bacillus*, *Carnobacterium*, staphylococci and streptococci.

Oxford agar

After 24 hours colonies of *Listeria* appear small, 1 mm in diameter, greyish surrounded by black halos (aesculin positive). After 48 hours colonies become darker, sometimes with a greenish sheen, and are about 2 mm in diameter with black halos and sunken centres

6.3.2 COUNTING OF COLONIES FROM THE ENUMERATION METHOD

For enumeration, use plates containing up to 150 colonies (if possible). If more than one colonial type is present on enumeration plates perform a differential count. If colonies with zones are present after 24 hours perform a count as zones may increase in size on further incubation and make counting difficult.

6.4 CONFIRMATORY TESTS

6.4.1 SELECTION OF COLONIES FOR CONFIRMATION

Sub-culture up to five presumptive *Listeria* colonies of each morphological type to horse blood agar from *Listeria* chromogenic agar (or all colonies if less than five are present) and perform confirmatory tests as described below.

If *L. monocytogenes* has not been detected (either through absence of typical colonies or through confirmatory tests yielding *Listeria* species other than *L. monocytogenes*), sub-culture five colonies (or all if less than five are present) from each of the sub-culture plates made from the primary and secondary enrichment broths. Examine carefully for different morphological appearances; if present sub-culture at least one representative of each type.

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If none of these colonies are confirmed as *L. monocytogenes*, but some or all of them are confirmed as *Listeria* species, a final count of *Listeria* species can be calculated (Section 7).

If the presence of *L. monocytogenes* has already been confirmed by enumeration, no further work need be carried out from the enrichment broth sub-culture plates unless an epidemiological investigation is being carried out in which a specific strain is sought.

6.4.2 CONFIRMATION

Sub-culture each presumptive *Listeria* colony selected to horse blood agar by first performing a single stab inoculation (to facilitate haemolysis detection) to give discrete colonies followed by separate streaking to demonstrate purity and to give discrete colonies. Incubate at 37°C for 24 ± 3 hours and examine for purity, colonial morphology and presence of β-haemolysis. Record the haemolysis results. Almost all strains of *L. monocytogenes* are haemolytic. Strains of *L. ivanovii* are strongly haemolytic and *L. seeligeri* are weakly haemolytic. Other species are non-haemolytic including *L. innocua*^{16,17}.

Information note: Some strains of *L. monocytogenes* rarely appear non-haemolytic on horse blood agar and if found should be sent to a reference laboratory with the result of the API identification system.

Select pure cultures of different morphological colony types for confirmation.

Perform a Gram stain if required to verify the nature of the isolates. *Listeria* species are Gram-positive, pleomorphic, non-sporing slender rods that are non-pigmented on horse blood agar.

For each morphological type perform biochemical testing with an API *Listeria* identification system following the manufacturer's instructions. Add the ZYM B reagent. *L. monocytogenes* gives a negative reaction in the DIM test but other species give a positive reaction¹⁷.

Information note: ZYM B reagent is sensitive to light and deteriorates rapidly, leading to false positive reactions. Store under refrigerated conditions, protect the reagent from light and minimise the length of time that the reagent is held at ambient temperature. Do not exceed the shelf life recommended by the manufacturer. Some laboratories have found that examination of DIM under UV light (365 nm wavelength) prior to addition of the ZYM B reagent helps to distinguish strains of *L. monocytogenes* from other *Listeria* species. Strains of *L. monocytogenes* do not fluoresce whilst other *Listeria* species will show blue/violet fluorescence due to the liberated naphthyl group (see section 3 for safety information regarding the use of ZYM B reagent). Appropriate precautions should be taken when working with UV light.

Acceptable API profiles are good, very good or excellent identification with a percentage of identification ≥ 90% and a T index ≥ 0.25.

Record the identity of the isolates. Also record the API profile, the percentage of identification and the T index.

6.4.4 QUALITY CONTROL ORGANISMS

The internal quality control procedures should be carried out following local protocols using the following controls:

Positive control:		
<i>Listeria monocytogenes</i>	NCTC 11994	
<i>Listeria innocua</i>	NCTC 11288	Control for <i>Listeria</i> chromogenic agar
Negative control:		
<i>Enterococcus faecalis</i>	NCTC 775	

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7 CALCULATION OF RESULTS

If enumeration has been performed, colony counts should be calculated where possible using dilutions yielding up to 150 colonies on the plate.

Calculate the number of *Listeria* species or *L. monocytogenes* per gram as follows:

$$\text{Count per g} = \frac{\text{Number of colonies confirmed}}{\text{Number of colonies tested}} \times \frac{\text{Number of colonies counted}}{\text{Volume tested} \times \text{dilution}}$$

8 REPORTING OF RESULTS

Report the count of all *Listeria* organisms including *L. monocytogenes* as *Listeria* species. If *L. monocytogenes* is detected, report this separately.

If *Listeria* species are not isolated by enrichment report as:

“*Listeria* species not detected in 25 g or in swab”

If *Listeria* species are not isolated by enumeration but are isolated by enrichment report as:

“*Listeria* species detected in 25 g (less than 10/g)”

Also report the identity of the species. If *L. monocytogenes* is not found, report this separately as described above.

If any colonies are confirmed as *Listeria monocytogenes* report as:

“*L. monocytogenes* detected in 25 g (less than 10/g)”

If *Listeria* species including *L. monocytogenes* are detected by enumeration, report the total count as *Listeria* species as a count per gram or mL. Also report the count of *L. monocytogenes* separately as a count per g or mL.

If the test organism is detected with counts between 10 and 99 per gram, report in the form of:

a cfu/g

where *a* is a number between 10 and 99

If the test organisms are detected at counts of greater than, or equal to, 100 per gram, report with one figure before and one figure after the decimal point expressed to the power of 10 in the form of;

a x 10^{*b*} cfu/g

where *a* is never less than 1.0 or greater than 9.9 and *b* represents the appropriate power of ten. Round counts up if the last figure is 5 or more and down if the last figure is 4 or less:

e.g: 1920 cfu /g reported as 1.9 x 10³ cfu/g
235,000 cfu/g reported as 2.4 x 10⁵ cfu/g

The actual weight of food examined must be reported, for example, 10 g, 25 g, 100 g.

DETECTION AND ENUMERATION OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA* SPECIES

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9 REFERENCE FACILITIES

All isolates of *L. monocytogenes* (haemolytic and non-haemolytic), regardless of the level should be sent to the Food Pathogens Reference Unit, Laboratory of Gastrointestinal Pathogens, HPA Centre for Infections for confirmation and further characterisation.

A request form for referral to reference facilities can be accessed at the following link <http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>.

Centre for Infections: tel: 0208 327 7116 or 0208 327 6505

10 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Food and Dairy Working Group for National Standard Methods (http://www.hpa-standardmethods.org.uk/wg_food_dairy_group.asp). The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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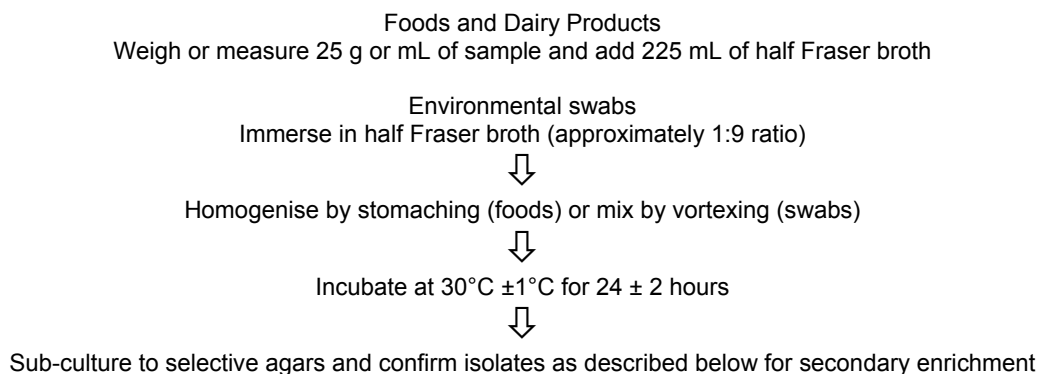
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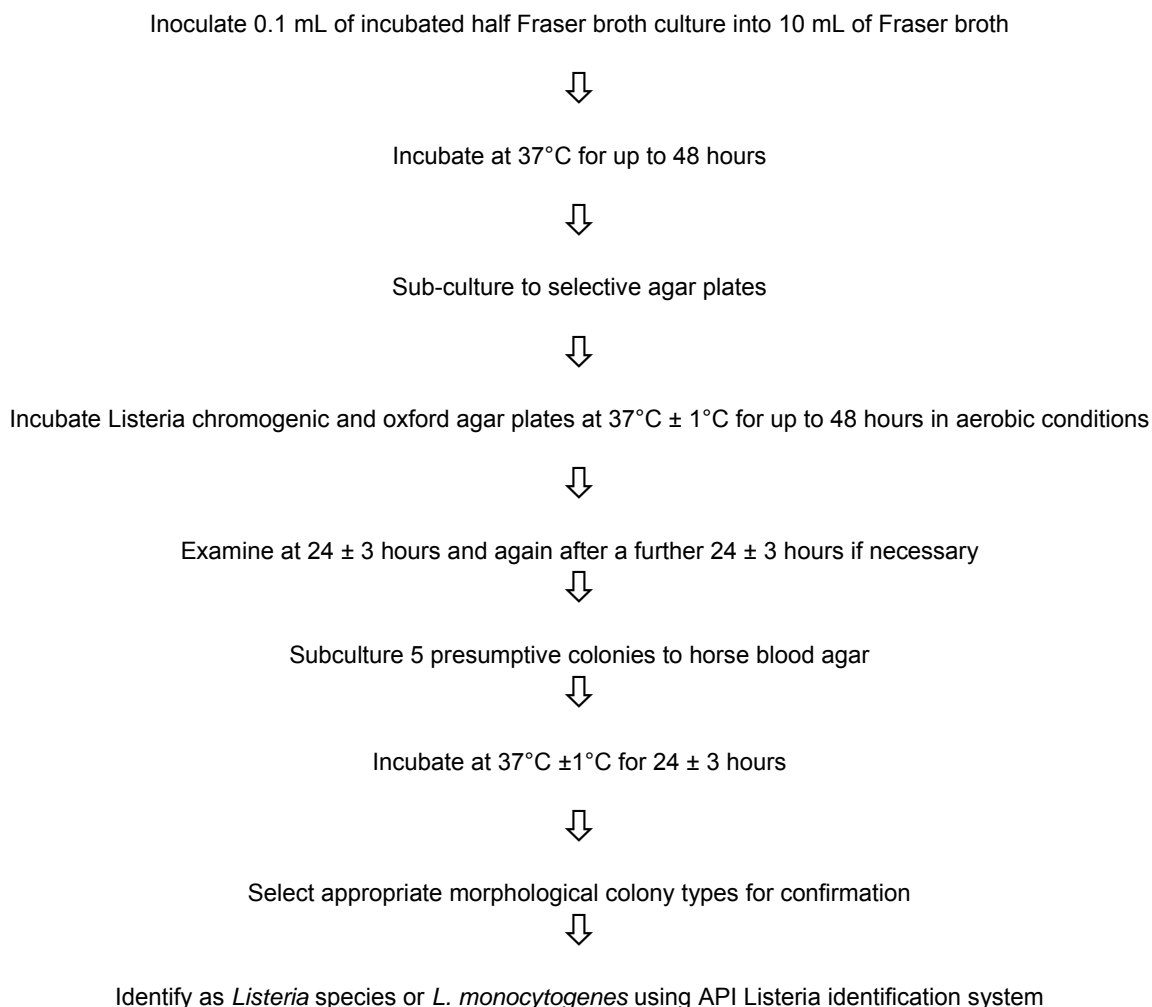
APPENDIX 1: FLOWCHARTS SHOWING THE PROCESS FOR THE DETECTION AND ENUMERATION OF LISTERIA MONOCYTOGENES AND OTHER LISTERIA SPECIES

Detection

Primary enrichment



Secondary enrichment



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Enumeration

Prepare a 10^{-1} dilution of sample



Homogenise by stomaching (food, swabs) or mix by vortexing (swabs)



Prepare further dilutions if required in peptone saline diluent



Surface spread 0.5 mL of 10^{-1} dilution on two Listeria chromogenic plates
If high counts are expected, also inoculate 50 μ L of a 10^{-1} and 10^{-3} dilution
onto Listeria chromogenic agar media using a spiral plater



Incubate Listeria chromogenic agar plates at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for up to 48 hours in aerobic conditions



Examine at 24 ± 3 hours and after a further 24 ± 3 hours.



Sub-culture 5 presumptive colonies onto horse blood agar



Incubate at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 ± 3 hours



Identify using API Listeria identification system



Calculate the counts of *Listeria* species (and *L. monocytogenes* if present) per gram or mL

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APPENDIX 2: COMPARISON OF THE HPA F19 METHOD AND ISO 11290 PARTS 1 AND 2

	HPA F19 method	ISO 11290 parts 1 and 2
Definition	Includes a definition of <i>Listeria</i> species	Only covers a definition for <i>Listeria monocytogenes</i>
Environmental swabs	Describes the processing for environmental swabs	Only applicable to products intended for human consumption or animal foodstuffs
Volume	Indicates the volume of the initial suspension to be applied to the agar for enumeration as 0.5 mL per 90 mm plate.	Indicates the volume of the initial suspension to be applied to the agar for enumeration is 0.1 mL (or 1 mL across three 140 mm plates).
Resuscitation of organisms	No resuscitation step included	Resuscitation step described
API Identification system	Describes the use of API identification system	ISO state identification steps
Reference laboratory	All isolates of <i>L. monocytogenes</i> to be sent for confirmation	All isolates may be sent for confirmation

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