

Making food safer according to ISO methods

Culture media and associated products for pathogen detection and enumeration



Introduction

The International Standards Organization (ISO) has published over 19,000 international standards that cover many different aspects of food testing.

Many companies choose to test human food, animal feed and environmental samples according to ISO methods. By safeguarding public health through the control of infectious organism levels, applying methods that conform to the standards set by accreditation bodies and regulatory authorities, companies are able to meet the increasing demands of their customers and maintain their reputation for supply of products that are safe to consume.

This guide describes the Thermo Scientific™ Microbiology products that conform to the formulations described in the top 16 most commonly used ISO standards for human food, animal feed and environmental samples.

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Part 1: Technique using Baird-Parker medium

Coagulase-positive Staphylococci

Staphylococcus aureus has implications in hygiene control, and also, as it produces enterotoxin, it is a key cause of food poisoning. Found most commonly in cheese, milk and in foods prepared by hand, its prevalence is widespread and of key importance to food manufacturers as it is a common bacterium found in the human nose and on the skin.

SAMPLE PREPARATION

As directed

ISOLATION

Surface inoculate 0.1mL of test sample or dilution onto

Baird Parker Medium (CM1127 + SR0054)

Or 1.0mL onto 1x140mm plate 3x90mm plates

Incubate for 24 hr \pm 2 hr at 35°C or 37°C

EXAMINE PLATES

Mark position of typical colonies

Incubate for 24 hr \pm 2 hr at 35°C or 37°C

CONFIRMATION

Brain Heart Infusion Broth (CM1135) Rabbit Plasma (R21050) Part 2: Technique using rabbit plasma fibrinogen medium

Coagulase-positive Staphylococci

Staphylococcus aureus has implications in hygiene control, and also, as it produces enterotoxin, it is a key cause of food poisoning. Found most commonly in cheese, milk and in foods prepared by hand, its prevalence is widespread and of key importance to food manufacturers as it is a common bacterium found in the human nose and on the skin.

ISOLATION

Duplicate pour plate using 1mL of test sample or dilution into

Rabbit Plasma Fibrinogen (RPF) (CM0961 + SR0122)

Incubate for 18–24 hr at 35°C or 37°C Incubate for a further 24 hr if required

REPORT RESULTS

Count typical colonies



Coagulase-positive Staphylococci

Staphylococcus aureus has implications in hygiene control, and also, as it produces enterotoxin, it is a key cause of food poisoning. Found most commonly in cheese, milk and in foods prepared by hand, its prevalence is widespread and of key importance to food manufacturers as it is a common bacterium found in the human nose and on the skin.

DETECTION METHOD

ENUMERATION METHOD

ENRICHMENT

Add Xg of test portion to 9XmL of s/s Giolitti and Cantoni Broth (CM0523)

OR

Add 10g of test portion to 10mL of d/s Giolitti and Cantoni Broth (CM0523)

Seal tube with agar or paraffin

Incubate for 24 hr \pm 2 hr at 37°C Reincubate negative tubes for up to 48 hr \pm 2 hr

Prepare dilutions in d/s and s/s

Giolitti and Cantoni Broth (CM0523 + SR0030) as directed

Incubate for 24 hr \pm 2 hr at 37°C Reincubate negative tubes for up to 48 hr \pm 2 hr

ISOLATION

OR

Subculture onto

Baird Parker Agar (CM1127 + SR0054)

Incubate for 24 hr \pm 2 hr at 37°C

Subculture onto

Rabbit Plasma Fibrinogen (RPF) Agar (CM0961 + SR0122)

Incubate for 24 hr ± 2 hr at 37°C

EXAMINE PLATES

Mark position of typical colonies

REPORT RESULTS

Count typical colonies

CONFIRMATION

Brain Heart Infusion Broth (CM1135) Rabbit Plasma (R21050)

Coagulase-positive Staphylococci

1.1 ISO 6888-1:1999

Product description	Product format	Product code
	Debudented Culture Media (CM)	CM1127B - 500g
Baird-Parker (ISO) Medium	Dehydrated Culture Media (CM)	CM1127T – 5Kg
	Petri Dish	P01195A
Egg Yolk Tellurite Emulsion	Bottle	SR0054C - 100mL
Egg Yolk Emulsion	Bottle	SR0047C - 100mL
Potassium Tellurite 3.5%	Tube	SR0030J – 10x2mL
		CM1135B - 500g
Brain Heart Infusion Broth	Dehydrated Culture Media (CM)	CM1135R – 2.5Kg
		CM1135T – 5Kg
Rabbit Plasma With EDTA		R21050 – 5mL
	Vial	R21051 – 15mL
	Viai	R21052 – 25mL
		R21060 – 6x5mL

1.2 ISO 6888-2:1999

Product description	Product format	Product code
	Bottle	B00290Y - 10x360mL
Baird Parker Agar Base (RPF)		B00290J – 10x90mL
		CM0961B - 500g
RPF Supplement	Vial	SR0122A - 10x100mL

1.3 ISO 6888-3:2003

Product description	Product format	Product code
	Debudrated Cultura Media (CM)	CM1127B - 500g
Baird-Parker (ISO) Medium	Dehydrated Culture Media (CM)	CM1127T – 5Kg
	Petri Dish	P01195A
Egg Yolk Tellurite Emulsion	Bottle	SR0054C - 100mL
Egg Yolk Emulsion	Bottle	SR0047C - 100mL
Potassium Tellurite 3.5%	Tube	SR0030J – 10x2mL
	Bottle	B00290Y - 10x360mL
Baird Parker Agar Base (RPF)	Dottie	B00290J – 10x90mL
	Dehydrated Culture Media (CM)	CM0961B - 500g
RPF Supplement	Vial	SR0122A – 10x100mL
Brain Heart Infusion Broth		CM1135B - 500g
	Dehydrated Culture Media (CM)	CM1135R - 2.5Kg
		CM1135T – 5Kg
		R21050
Rabbit Plasma With FDTA	Vit/Paggent	R21051
Raddit Plasma with Edia	Kit/Reagent	R21052
		R21060
Giolitti and Cantoni Broth	Debudrated Cultura Madia (CM)	CM0523B - 500g
dionili and Cantoni Broun	Dehydrated Culture Media (CM)	CM0523R - 2.5Kg

Thermo Scientific Brilliance Staph 24

Thermo Scientific[™] *Brilliance*[™] Staph 24 Agar—a selective and diagnostic chromogenic medium for the isolation and enumeration of coagulase-positive Staphylococci in foods, within 24 hours.

OBSERVATION MADE SIMPLE

• Dark blue colonies on a clear background

RAPID RESULTS

Enumeration in just 24 hours

DEFINITIVE ANSWERS

- Detects coagulase-positive Staphylococci, including pathogenic coagulase-positive, non-aureus Staphylococci, such as S. intermedius
- Prevents growth of nontarget organisms, therefore, eliminating extensive confirmatory testing and miscalculation of cell counts

CONFIDENT CONCLUSIONS

ISO 16140 validated

ISO 16140 Validation

The Thermo Scientific *Brilliance* Staph 24 Agar method has been validated and approved by MicroVal according to ISO 16140 Standard against the reference method ISO 6888:1999-Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species) — Part 1: Technique using Baird-Parker Agar for all human food products. MicroVal certificates are available in PDF format from www.microval.org.

Protocol for enumeration of coagulase-positive Staphylococci using *Brilliance* Staph 24

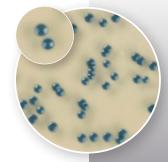
Plating

Dilute sample in appropriate diluent

Plus

In duplicate, spread 0.1mL of appropriate dilution onto 2x *Brilliance* Staph 24 Agar plates

Incubate for 24 hr \pm 2 hr at 37°C \pm 1°C



Results

If present, select 5 well isolated dark blue colonies for use in confirmation



Horizontal method for the detection of

Salmonella species

The genus Salmonella belongs to the family Enterobacteriaceae. Salmonella bacteria are Gram-negative, non spore forming rods. There are approximately 2,500 serovars of Salmonella, which are characterized according to somatic and flagella antigens. Salmonella is one of the most frequent causes of food poisoning and a major public health problem worldwide. 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.

PRE-ENRICHMENT

Prepare 1:10 dilution in Buffered Peptone Water (ISO) (CM1049) at ambient temperature

Incubate for 18 hr \pm 2 hr at 37°C \pm 1°C

SELECTIVE ENRICHMENT

0.1mL of culture in 10mL of RVS Broth (CM0866)

Incubate for 24 hr \pm 3 hr at 41.5°C \pm 1°C

1mL of culture in 10mL of MKTTn Broth (CM1048 + SR0181)

Incubate for 24 hr \pm 3 hr at 37°C \pm 1°C

ISOLATION

XLD Agar (CM0469) plus second agar of choice e.g. *Brilliance* Salmonella Agar (CM1092 + SR0194) Brilliant Green Agar (mod) (CM0329)

Incubate for 24 hr \pm 3 hr at 37°C \pm 1°C

PURITY PLATE

Nutrient Agar

Incubate for 24 hr \pm 3 hr at 37°C \pm 1°C

BIOCHEMICAL CONFIRMATION

TSI Agar (CM0277) Urea Agar (CM0053 + SR0020)

L-Lysine Decarboxylation Medium
Detection of β-galactosidase
Voges-Proskauer ReactionMRVP (CM0043)

Indole Reaction—Tryptone Water (CM0087) + Kovac's Reagent (MB0209A)

Physiological saline

SEROLOGICAL CONFIRMATION

O-antigens (R30858201) Vi-antigens (R30957401) H-antigens (R30858501)



Salmonella species

2.1 ISO 6579:2002

Product description	Product format	Product code
	Dottle	B01067S - 10x225mL
	Bottle	B01067Z – 10x950mL
		CM1049B - 500g
	Dehydrated Culture Media (CM)	CM1049R - 2.5Kg
B ((a) B		CM1049T – 5Kg
Buffered Peptone Water (ISO)	D. D. IM	DB1049W
	Dry-Bag [™]	DB1049M
	ReadyBag	BM1104T
	Tube	TV5013D
	Universal	B01067E
		CM0866B - 500g
	Dehydrated Culture Media (CM)	CM0866R - 2.5Kg
RVS Broth		CM0866K – 25Kg
RVS BIOUI	Tube	TV5036E
	Heirard	EB0499E
	Universal	EB0499M
	Bottle	B01224K – 10x50mL
MKTTn Broth	Dehydrated Culture Media (CM)	CM1048B - 500g
	Tube	TV5065E
Novobiocin Supplement	Vial	SR0181E
		CM0469B - 500g
	Dehydrated Culture Media (CM)	CM0469R - 2.5Kg
XLD Agar		CM0469T – 5Kg
	Date: Diah	P00164A
	Petri Dish	P05057A
Trinla Curar Iran Asar (TCI)	Dehydrated Culture Media (CM)	CM0277B - 500g
Triple Sugar Iron Agar (TSI)	Tube	TV5074D
	Dehydrated Culture Media (CM)	CM0053B - 500g
Urea Agar	Clana	B00337B – 24x3mL
	Slope	EB0337B – 200x3mL
Urea 40% Solution	Vial	SR0020K
MRVP Medium	Dehydrated Culture Media (CM)	CM0043B - 500g
		B00383B
Tryptone Water	Bijou	B00383C
i i yptolie watei		EB0383B
	Dehydrated Culture Media (CM)	CM0087B - 500g
Kovac's Reagent	Bottle	MB0209A
Salmonella 0 agglutinating Sera	Kit/Reagent	R30858201
Salmonella Vi agglutinating Sera	Kit/Reagent	R30957401
Salmonella H agglutinating Sera	Kit/Reagent	R30858501

Salmonella species

Second Mediums of choice: ISO 6579:2002

Product description	Product format	Product code
		CM0329B - 500g
	Dehydrated Culture Media (CM)	CM0329R - 2.5Kg
Drilliant Croop Ager (modified)	Denydrated Culture Media (CM)	CM0329T - 5Kg
Brilliant Green Agar (modified)		CM0329K - 25Kg
	Petri Dish	P00171A
		P05033A
	Dehydrated Culture Media (CM)	CM1092B - 500g
Brilliance Salmonella Agar Base	Denydrated Culture Media (CM)	CM1092T – 5Kg
	Petri Dish	P05098A
Brilliance Salmonella/XLD Bi-plate	Petri Dish	P05248E
Brilliance Salmonella Selective Supplement	Vial	SR0194E

Salmonella Precis-ISO 16140 Alternate method validated against ISO 6579:2002

Product description	Product format	Product code
	Debuggeted Culture Medic (CM)	CM1092B - 500g
Brilliance Salmonella Agar Base	Dehydrated Culture Media (CM)	CM1092T – 5Kg
	Petri Dish	P05098A
Brilliance Salmonella Selective Supplement	Vial	SR0194E
	Bottle	B01096S - 10x225mL
	Debydrated Culture Media (CM)	CM1091B - 500g
ONF Broth-Salmonella Base	Dehydrated Culture Media (CM)	CM1091T – 5Kg
JNE BIUIII-Saiiiioilella base	PoodyPog	FR60481
	ReadyBag	FR60101
	Dry-Bag	DB1091W
ONE Proth Colmonalla Coloctiva Cumplement	VE-1	SR0242E – 225mL
ONE Broth-Salmonella Selective Supplement	Vial	SR0242B - 2.25L
Salmonella Latex Test	Kit/Reagent	FT0203A
Oxoid Salmonella Latex Kit	Kit/Reagent	DR1108A

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 by AFNOR Certification to ISO 16140 standard
- Simple procedure—no specialised equipment required
- Single 18-hour enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Quick and convenient confirmation: Thermo Scientific™ Oxoid™ Salmonella Latex Test or ISO 6579:2002 standard tests
- Reduced time to result: 2 days compared with up to 5 days for standard culture methods
- Thermo Scientific™ Brilliance™ Salmonella Agar contains novel Thermo Scientific™ Inhibigen™ technology, giving targeted specificity and reduced background flora

AFNOR Validation

The Salmonella Precis[™] method has been validated and approved by AFNOR Certification according to ISO 16140 Standard against the reference method ISO 6579:2002 Standard for the detection of Salmonella in food, animal feed and environmental samples, excluding breeding samples.

For flexibility, confirmation was validated using both Salmonella Latex Test and the tests outlined in ISO 6579:2002. Alternatively, biochemical panels such as Thermo Scientific[™] Microbact[™] GNB 24E or Thermo Scientific[™] RapID ONE[™] Panel, may be used.

AFNOR Certification validation certificate (available in PDF format from the AFNOR website www.afnor-validation.com).

Reactions on *Brilliance*™ Salmonella Agar

	Colony colour/appearance		
	Purple	Blue	Colourless
Enzyme targeted by chromogen	Salmonella (including Lactose positive Salmonella)	Klebsiella, Enterobacter, Serratia	Citrobacter, other bacteria and yeasts
Esterase	+	-/+	-
β-glucosidase	-	+	-

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





Day 0: Enrichment

25g or 25mL of sample + 225mL ONE Broth-Salmonella

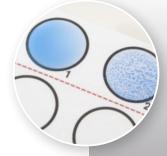
Incubate for 16 - 20 hr at 42°C



Day 1: Plating

Using a 10µL microbiological loop inoculate a single *Brilliance* Salmonella Agar plate

Incubate for 20 – 26 hr at 37°C



Day 2: Results

If present, select a well isolated purple coloured colony and test using the Oxoid Salmonella

Alternatively, confirm purple colonies using standard ISO methods

Select purple colonies for confirmation

Part 1: Detection method

Listeria monocytogenes

Listeria are Gram-positive, catalase positive, non spore forming rods with flagella. *Listeria monocytogenes* and *Listeria ivanovii* are consistently associated with human illness isolated from soil, vegetation and water. With growth temperature from 0°C to 45°C, it is a key foodborne pathogen in chilled, refrigerated and ready-to-eat foods.



Add Xg or XmL of sample/dilution to 9XmL of Half Fraser Broth (CM0895 + SR0166)

Incubate for 24 hr ± 2 hr at 30°C

SECONDARY ENRICHMENT

Transfer 0.1mL of primary enrichment culture to 10mL of Fraser Broth (CM0895 + SR0156)

Incubate for 48 hr ± 2 hr at 35°C or 37°C

Incubate for 24 hr \pm 2 hr at 30°C

SELECTIVE ISOLATION

Inoculate onto ALOA™ OCLA (ISO) (CM1084 + SR0244, SR0226) and one other medium of choice Second medium of choice: PALCAM (CM0877 + SR0150), Oxford (CM0856 + SR0140 or SR0206)

Incubate for 24 hr \pm 3 hr at 37°C and, if necessary, for an additional 24 hr \pm 3 hr

PURITY PLATE

TSYEA-Tryptone Soya Yeast Extract Broth (CM0862 + 9–18g Agar)

Incubate for 18-24 hr at 35°C-37°C

CONFIRMATION OF LISTERIA SPP.

Catalase Test Gram Stain Motility Test: Tryptone Soya Yeast Extract Broth (CM0862)

s/s = single strength d/s = double strength XmL = sample size Xg = sample size

CONFIRMATION OF LISTERIA MONOCYTOGENES

Haemolysis Test: Sheep Blood Agar (B00965) Carbohydrate Utilization CAMP Test



Part 2: Enumeration method

Listeria monocytogenes

Listeria are Gram-positive, catalase positive, non spore forming rods with flagella. *Listeria monocytogenes* and *Listeria ivanovii* are consistently associated with human illness isolated from soil, vegetation and water. With growth temperature from 0°C to 45°C, it is a key foodborne pathogen in chilled, refrigerated and ready-to-eat foods.



Add Xg or XmL of sample to 9XmL of Buffered Peptone Water (ISO) (CM1049) or Half Fraser Broth (CM0895 + SR0166)

Allow suspension to stand for 1 hr \pm 5 min at 20°C \pm 2°C

SELECTIVE ISOLATION

Inoculate onto ALOA™

OCLA (ISO) Agar (CM1084 + SR0244, SR0226)

Incubate for 24 hr \pm 3 hr at 37°C

PURITY PLATE

TSYEA-Tryptone Soya Yeast Extract Broth (CM0862 + 9–18g Agar)

Incubate for 18-24 hr at 37°C

CONFIRMATION OF LISTERIA SPP.

Catalase Test Gram Stain Motility Test: Tryptone Soya Yeast Extract Broth (CM0862)

CONFIRMATION OF LISTERIA MONOCYTOGENES

Haemolysis Test: Sheep Blood Agar (B00965)
Carbohydrate Utilization
CAMP Test

Listeria monocytogenes

3.1 ISO 11290-1:1996

Product description	Product format	Product code
	Bottle	B00407E – 24x10mL
		CM0895B - 500g
Fraser Broth Base	Dehydrated Culture Media (CM)	CM0895R - 2.5Kg
		CM0895T – 5Kg
	Tube	TV5020E
Fraser Supplement	Vial	SR0156E
Fraser Broth + Supplement	Bottle	B01034E – 24x10mL
Traser Brour + Supplement	Dottie	EB1034E – 100x10mL
Half Fraser Supplement	Vial	SR0166E – 225mL
Hall Hasel Supplement	Viai	SR0166G – 2.25L
		B00350S - 10x225mL
		B00350V – 10x500mL
	Bottle	B00350Z – 10x450mL
Half Fraser + Supplement		B00793S - 10x225mL
Trail Traser + Supplement		B00350J – 10x90mL
	Dry-Bag	DB0895V
	Dry-bay	DB0895L
	ReadyBag	FR59562
	Dehydrated Culture Media (CM)	CM1084B - 500g
Oxoid Chromogenic Listeria Agar (OCLA) (ISO)	Deriyurated Guiture Media (GM)	CM1084R – 2.5Kg
	Petri Dish	P01196A
OCLA (ISO) Selective Supplement	Vial	SR0226E
OCLA (ISO) Differential Supplement	Vial	SR0244E
		CM0862B - 500g
Listeria Enrichment Broth Base	Dehydrated Culture Media (CM)	CM0862R - 2.5Kg
		CM0862T – 5Kg
Defibrinated Sheep Blood	Vial	SR0051B
	Bottle	B00965Z – 10x450mL
lood Agar No. 2 + Sheep Blood	DULLIC	B00965M – 10x100mL
	Petri Dish	PB0115A

Listeria monocytogenes

3.2 Second Mediums of choice: ISO 11290-1:1996

Product description	Product format	Product code
		CM0877B - 500g
PALCAM Agar	Dehydrated Culture Media (CM)	CM0877R - 2.5Kg
PALCAIVI Agai		CM0877T – 5Kg
	Petri Dish	P05104A
PALCAM Selective Supplement	Vial	SR0150E – 500mL
PALCAIN Selective Supplement		SR0150B – 2.5L
		CM0856B - 500g
Listeria Selective Agar (Oxford Formulation)	Dehydrated Culture Media (CM)	CM0856R - 2.5Kg
Listeria Selective Agai (Oxiora Formulation)		CM0856T – 5Kg
	Petri Dish	P05026A
Oxford Selective Supplement	Vial	SR0140E
Modified Oxford Supplement	Vial	SR0206E

Listeria Precis – ISO 16140 Alternate method validated against ISO 11290-1:1996

Product description	Product format	Product code
		CM1080B - 500g
	Dehydrated Culture Media (CM)	CM1080T – 5Kg
Brilliance Listeria Agar Base		CM1080E – 2L pack
	Petri Dish	P01102A
	Petil Disti	P05165A
Brilliance Listeria Selective Supplement	Vial	SR0227E
Brilliance Listeria Differential Supplement	Vial	SR0228E
	Bottle	B01066S – 10x225mL
	Dehydrated Culture Media (CM)	CM1066B - 500g
ONF Broth-Listeria Base		CM1066R - 2.5Kg
ONE DIOUT-LISTETIA DASE		CM1066T – 5Kg
	ReadyBag	FR60031
	Dry-Bag	DB1066V
		SR0234E – 500mL
ONE Broth-Listeria Supplement	Vial	SR0234H – 2L
		SR0234B - 2.25L
0.B.I.S. Listeria	Kit/Reagent	ID0600M
Microbact 12L	Kit/Reagent	MB1128A
Microbact 12L Haemolysin Reagent	Kit/Reagent	MB1249A

Listeria monocytogenes

ISO 11290-2:1998

Product description	Product format	Product code
	D. III.	B01067S – 10x225mL
	Bottle	B01067Z – 10x950mL
		CM1049B - 500g
	Dehydrated Culture Media (CM)	CM1049R - 2.5Kg
		CM1049T – 5Kg
Buffered Peptone Water (ISO)	Dry Dog	DB1049W
	Dry-Bag	DB1049M
	ReadyBag	BM1104T
	Tube	TV5013D
	Universal	B01067E
		B01071E
Oxoid Chromogenic Listeria Agar (OCLA)	Debuduated Cultura Media (CM)	CM1084B - 500g
(ISO) Base	Dehydrated Culture Media (CM)	CM1084R – 2.5Kg
OCLA (ISO) Selective Supplement	Vial	SR0226E
OCLA (ISO) Differential Supplement	Vial	SR0244E
Oveid Chromogonia Listoria (OCLA) (ICO) Ager	Petri Dish	P01196A
Oxoid Chromogenic Listeria (OCLA) (ISO) Agar		P05183A
Listeria Enrichment Broth Base (TSYEB formulation)	Dehydrated Culture Media (CM)	CM0862B - 500g
		CM0862R - 2.5Kg
		CM0862T - 5Kg
Defibrinated Sheep Blood	Vial	SR0051B
Blood Agar No. 2 + Sheep Blood	Petri Dish	PB0115A

Listeria monocytogenes

Listeria Precis – ISO 16140 Alternate method validated against ISO 11290 part 1:1996 and part 2:1998

Product description	Product format	Product code
		CM1080B - 500g
	Dehydrated Culture Media (CM)	CM1080T – 5Kg
Brilliance Listeria Agar Base		CM1080E – 2L pack
	Dotri Diah	P01102A
	Petri Dish	P05165A
Brilliance Listeria Selective Supplement	Vial	SR0227E
Brilliance Listeria Differential Supplement	Vial	SR0228E
	Bottle	B01066S - 10x225mL
	Dehydrated Culture Media (CM)	CM1066B - 500g
ONF Broth-Listeria Base		CM1066R - 2.5Kg
ONE DIOUI-LISTERIA DASE		CM1066T - 5Kg
	ReadyBag	FR60031
	Dry-Bag	DB1066V
		SR0234E - 500mL
ONE Broth-Listeria Supplement	Vial	SR0234H – 2L
		SR0234B - 2.25L
O.B.I.S. Listeria	Kit/Reagent	ID0600M
Microbact 12L	Kit/Reagent	MB1128A
Microbact 12L Haemolysin Reagent	Kit/Reagent	MB1249A

Listeria Precis Method

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

- Validated by AFNOR Certification to ISO 16140 standard
- Simple procedure—no specialised equipment required
- Single 24-hour enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Quick and convenient confirmation: O.B.I.S. mono test or ISO 11290 standard tests
- Reduced time to result: 2 days compared with up to 7 days for standard culture and confirmation

AFNOR Validation

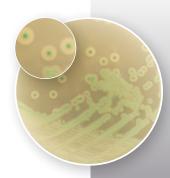
The Listeria Precis[™] method has been validated and approved by AFNOR according to ISO 16140 Standard against the reference methods ISO 11290 Part 1:1997 and Part 2:1997 incorporating Amendment 1:2004 for the detection and enumeration of *L. monocytogenes* in food and environmental samples. AFNOR Certification validation certificates are available in PDF format from the AFNOR Certification website www.afnor-validation.com.

For flexibility, confirmation was validated using either the O.B.I.S. mono test or tests outlined in ISO 11290. Alternatively, biochemical panels, such as $\mathsf{Microbact}^\mathsf{TM}$ 12L or Thermo Scientific TM Rapl D^TM CB Plus Panel, may be used.

	Colony colour/appearance		
	Blue	Blue + halo	Colourless or inhibited
Enzyme targeted	Listeria spp.	L. monocytogenes and pathogenic L. ivanovii	Non-Listeria
β-glucosidase	+	+	-
Lecithinase	-	+	-

The O.B.I.S. mono test allows rapid differentiation of *L. monocytogenes* from other Listeria species. All Listeria species, with the exception of *L. monocytogenes*, possess the enzyme D-alanyl aminopeptidase. Its presence can be detected using the substrate, D-alanyl-7-amido-4-methylcoumarin (DALA), and colour developer, dimethylamino-cinnamaldehyde. O.B.I.S. mono produces a deep purple reaction if this enzyme is present.





Day 1: Plating

Using a 10µL microbiological loop inoculate a single *Brilliance* Listeria Agar plate

Incubate for 22 - 26 hr at 37°C

Select green/blue colonies with halos for confirmation

(for meat samples re-incubate plates that show no blue/green colonies with halos for a further 22-26 hr at 37°C)



Day 2: Results

If present, confirm blue/ green colonies with halos as *L. monocytogenes* using the O.B.I.S. mono test

Alternatively, confirm using standard ISO methods**

For *Listeria monocytogenes* enumeration:

Resuscitate any organisms present in the sample by adding 25g or 25mL to 225mL of Buffered Peptone Water and incubate for 1 hour at 20°C. Inoculate a single *Brilliance* Listeria plate with 100µL and incubate for 45 to 51 hours at 37°C. Inspect the plate for characteristic blue/green colonies with halos and count. Confirm using 0.B.I.S. mono or alternatively, confirm using standard ISO methods.** Calculate CFU/g or CFU/mL of sample.

**If there is insufficient material to carry out an O.B.I.S. mono test, or if a mixed culture of *L. monocytogenes* and other Listeria species is suspected, first purify suspect colonies by sub-culture onto a second *Brilliance* Listeria plate.

Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

β-glucuronidase-positive Escherichia coli

Escherichia coli are a member of the family Enterobacteriaceae, and are divided into many sub-groups. E. coli is used as an indicator organism in water testing for the presence of faecal coliforms. A persistent cause of diarrheagenic pathogenicity from uncooked food, the most significant group based on severity of illness is E. coli EHEC, which includes the 0157: H7 strain.



RESUSCITATION

1mL of sample/dilution onto membrane on Minerals Modified Glutamate Agar

Incubate for 4 hr ± 1 hr at 37°C

ISOLATION

Transfer membrane to Tryptone-Bile-Glucuronic Agar

TBX Medium (CM0945)

Incubate for 18-24 hr at 44°C Not more than 4 plates high

REPORT RESULTS

Count typical colonies

Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

β-glucuronidase-positive Escherichia coli

Escherichia coli are a member of the family Enterobacteriaceae, and are divided into many sub-groups. *E. coli* is used as an indicator organism in water testing for the presence of faecal coliforms. A persistent cause of diarrheagenic pathogenicity from uncooked food, the most significant group based on severity of illness is *E. coli* EHEC, which includes the 0157: H7 strain.



ISOLATION

Pour plates in duplicate using 1mL of test sample/dilution and Tryptone-Bile-Glucuronic Agar TBX Medium (CM0945) CM0945

Incubate for 18-24 hr at 44°C

OR

Incubate for 4 hr at 37°C followed by 18–24 hr at 44°C (if stressed cells are suspected)

REPORT RESULTS

Count typical colonies

Part 3: MPN technique using 5-bromo-4-chloro-3-indolyl- β-D-glucuronide

β-glucuronidase-positive Escherichia coli

Escherichia coli are a member of the family Enterobacteriaceae, and are divided into many sub-groups. *E. coli* is used as an indicator organism in water testing for the presence of faecal coliforms. A persistent cause of diarrheagenic pathogenicity from uncooked food, the most significant group based on severity of illness is *E. coli* EHEC, which includes the 0157: H7 strain.



MPN

Add 10mL of liquid sample or initial suspension to each of 3 or 5 tubes of 10mL of Double strength Minerals Modified Glutamate Broth (CM0607)

Incubate for 24 hr \pm 2 hr at 37°C

AND

Add 1mL of liquid sample or initial suspension to each of 3 or 5 tubes of 10mL of Minerals Modified Glutamate Broth (CM0607)

Proceed in the same way with further dilutions

Incubate for 24 hr \pm 2 hr at 37°C

CONFIRMATION

Inoculate tubes showing acid production onto
Tryptone-Bile-Glucuronic Agar TBX Medium (CM0945)

Incubate for 22 hr ± 2 hr at 44°C Not more than 3 plates high

CALCULATE MPN

Look for typical blue colonies

β-glucuronidase-positive Escherichia coli

- 4.1 ISO 16649-1:2001
- 4.2 ISO 16649-2:2001
- 4.3 ISO 16649-3:2005

Product description	Product format	Product code
TBX Medium	Bottle	B00194M - 10x100mL
	Dehydrated Culture Media (CM) Petri Dish	CM0945B - 500g
		CM0945R - 2.5Kg
		CM0945T – 5Kg
		P00727A
	Petii Disii	P05109A

4.3 ISO 16649-3:2005

Product description	Product format	Product code
Minerals Modified Glutamate Broth	Dehydrated Culture Media (CM)	CM0607B - 500g
Sodium Glutamate	Dehydrated Culture Media (CM)	LP0124



presumptive Escherichia coli

Most probable number technique

Escherichia coli are a member of the family Enterobacteriaceae, and are divided into many sub-groups. *E. coli* is used as an indicator organism in water testing for the presence of faecal coliforms. A persistent cause of diarrheagenic pathogenicity from uncooked food, the most significant group based on severity of illness is *E. coli* EHEC, which includes the 0157: H7 strain.

SELECTIVE ENRICHMENT

Add 1mL of initial suspension to 9mL s/s Lauryl Sulphate Broth (CM0451)

OR

Add 10mL of initial suspension to 10mL d/s Lauryl Sulphate Broth (CM0451)

†Modifications apply for larger samples

Incubate for 24 hr \pm 2 hr at 37°C Incubate negative tubes for up to 48 hr \pm 2 hr

PRE-ENRICHMENT

Prepare dilutions in d/s and s/s

Lauryl Sulphate Broth (CM0451)

Incubate for 24 hr \pm 2 hr at 37°C Incubate negative tubes for up to 48 hr \pm 2 hr

SELECTIVE ENRICHMENT OF POSITIVE CULTURES

Inoculate one loopful of culture into EC Broth (CM0853)

Incubate for 24 hr \pm 2 hr at 44°C Incubate negative tubes for up to 48 hr \pm 2 hr

Inoculate one loopful of culture into EC Broth (CM0853)

Incubate for 24 hr \pm 2 hr at 44°C Incubate negative tubes for up to 48 hr \pm 2 hr

CONFIRMATION OF POSITIVE CULTURES

Inoculate one loopful of EC Broth culture into Tryptone Water (CM0087)

Incubate for 48 hr \pm 2 hr at 44°C

Inoculate one loopful of EC Broth culture into Tryptone Water (CM0087)

Incubate for 48 hr \pm 2 hr at 44°C

TEST FOR INDOLE PRODUCTION

Add Indole Reagent (MB0209A) and examine for red colour

Add Indole Reagent (MB0209A) and examine for red colour

s/s = single strength d/s = double strength XmL = sample size Xg = sample size

Calculate MPN

presumptive Escherichia coli

Most probable number technique

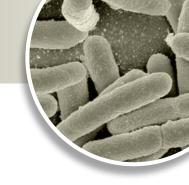
5 ISO 7251:1005

Product description	Product format	Product code
		CM0451B - 500g
La cal O Ashata Tarata a Basili	Dehydrated Culture Media (CM)	CM0451R - 2.5Kg
Lauryl Sulphate Tryptose Broth		CM0451T – 5Kg
	Tube	TV5201G
EC Broth	Dehydrated Culture Media (CM)	CM0853B - 500g
	Bijou	B00383B
Tryptone Water		B00383C
i ryptone water		EB0383B
	Dehydrated Culture Media (CM)	CM0087B - 500g
Indole Reagent	Kit/Reagent	MB0209A
Rapid Spot Indole	Kit/Reagent	R8309002

Horizontal method for the detection of

Escherichia coli 0157

Escherichia coli are a member of the family Enterobacteriaceae, and are divided into many sub-groups. *E. coli* is used as an indicator organism in water testing for the presence of faecal coliforms. A persistent cause of diarrheagenic pathogenicity from uncooked food, the most significant group based on severity of illness is *E. coli* EHEC, which includes the 0157: H7 strain.



SELECTIVE ENRICHMENT

Add Xg or XmL of sample/dilution to 9XmL of Modified Tryptone Soya Broth with Novobiocin (CM0989 + SR0181)

Incubate for 6 hr at 41.5°C and for a further 12-18 hr

IMMUNOCAPTURE

Using immunomagnetic separation

SELECTIVE ISOLATION

Inoculate onto CT-SMAC (CM0813 + SR0172) and one other medium of choice e.g. CR-SMAC (CM1005 + SR0191)

Incubate for 18-24 hr at 37°C

PURITY PLATE

Nutrient Agar

Incubate for 18-24 hr at 37°C

CONFIRMATION

Indole formation: Tryptone Water (CM0087 + MB0209A)
Serology: E.coli 0157 anti-sera
E.coli 0157 Latex: (DR0620M)

Escherichia coli 0157

6 ISO 16654:2001

Product description	Product format	Product code
	Bottle	B01078S - 10x225mL
Modified TSB	Debuggated Culture Media (CM)	CM0989B - 500g
	Dehydrated Culture Media (CM)	CM0989R - 2.5Kg
Novobiocin Supplement	Vial	SR0181E
Cefixime Tellurite Sorbitol MacConkey Agar	Debudrated Culture Media (CM)	CM0813B - 500g
(C-T SMAC) Base	Dehydrated Culture Media (CM)	CM0813R - 2.5Kg
Cefixime Tellurite Selective Supplement	Vial	SR0172E
centime remarke selective supplement	Viai	SR0172H
C T SMAC Agor	Petri Dish	P00702A
C-T SMAC Agar		P05069A
	Bijou	B00383B
Tryptone Water		B00383C
Tryptone water		EB0383B
	Dehydrated Culture Media (CM)	CM0087B - 500g
DrySpot E. coli 0157	Kit/Reagent	DR0120M
E. coli 0157 Latex Test	Kit/Reagent	DR0620M
Indole Reagent	Kit/Reagent	MB0209A
Rapid Spot Indole	Kit/Reagent	R8309002

Second Mediums of choice: 16654:2001

Product description	Product format	Product code
C-Rhamnose SMAC Agar	Dehydrated Culture Media (CM)	CM1005B - 500g
	Dehydrated Culture Media (CM)	CM0956A - 100g
Brilliance E. coli / coliform Agar		CM0956B - 500g
		CM0956R - 2.5Kg
	Petri Dish	P00745A
Cefixime Supplement	Vial	SR0191E

Part 1: Detection and enumeration by MPN with pre-enrichment

Enterobacteriaceae

The Enterobacteriaceae family of Gram-negative bacteria includes *Salmonella, Escherichia, Klebsiella, Shigella, Proteus, Enterobacter, Serratia,* and *Citrobacter.* These families of bacteria are of importance to food manufacturers as many members of this family are a normal part of the human gut flora and gut flora of other animals. Enterobacteriaceae are also found in water or soil and as such are used as key indicator organisms to determine the presence of more virulent and pathogenic bacteria, as well as an indicator of poor manufacturing hygiene, disrupted processes or contaminated environments.

DETECTION METHOD

MPN METHOD

PRE-ENRICHMENT

Add Xg or XmL of sample to 9XmL of Buffered Peptone Water (ISO) (CM1049)

Incubate for 18 hr \pm 2 hr at 37°C

Prepare dilutions using Buffered Peptone Water (ISO) (CM1049)

Incubate for 18 hr ± 2 hr at 37°C

SELECTIVE ENRICHMENT

Add 1mL of culture to 10mL of EE Broth (CM0317)

Incubate for 24 hr \pm 2 hr at 37°C

Add 1mL of culture to 10mL of EE Broth (CM0317)

Incubate for 24 hr ± 2 hr at 37°C

ISOLATION

Surface inoculate

VRBGA (ISO) (CM1082)

Incubate for 24 hr ± 2 hr at 37°C

Surface inoculate VRBGA (ISO) (CM1082)

Incubate for 24 hr ± 2 hr at 37°C

PURITY PLATE

Nutrient Agar

Incubate for 24 hr \pm 2 hr at 37°C

Nutrient Agar

Incubate for 24 hr ± 2 hr at 37°C

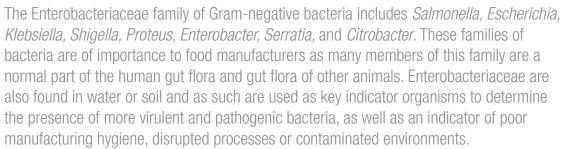
CONFIRMATION

Oxidase Test (R21540) Fermentation of glucose Oxidase Test (R21540) Fermentation of glucose

s/s = single strength d/s = double strength XmL = sample size Xg = sample size

Part 2: Colony-count method

Enterobacteriaceae





ISOLATION

Pour plate in duplicate of 1mL of test sample/dilution in VRBGA (ISO) (CM1082) with overlay

Incubate for 24 hr ± 2 hr at 37°C

COUNT

Count typical colonies

PURITY PLATE

Nutrient Agar

Incubate for 24 hr \pm 2 hr at 37°C

BIOCHEMICAL CONFIRMATION

Oxidase Test (R21540) Fermentation of glucose

Enterobacteriaceae

7.1 ISO 21528-1:2004

Product description	Product format	Product code
	Bottle	B01067S – 10x225mL
	bottle	B01067Z – 10x950mL
		CM1049B - 500g
	Dehydrated Culture Media (CM)	CM1049R - 2.5Kg
D. ((D W. (100)		CM1049T – 5Kg
Buffered Peptone Water (ISO)	Dry Pog	DB1049W
	Dry-Bag	DB1049M
	ReadyBag	BM1104T
	Tube	TV5013D
	Universal	B01067E
	Ulliversal	B01071E
		B00443Z – 10x90mL
	Bottle	B00598M – 10x100mL
		B00076M - 10x100mL
EE Broth		CM0317B - 500g
EE Broth	Dehydrated Culture Media (CM)	CM0317R - 2.5Kg
		CM0317T - 5Kg
	Tube	TV5041E
	Universal	B00598E
VRBGA (ISO)	Dehydrated Culture Media (CM)	CM1082B - 500g
Oxidase Test	Kit/Reagent	R21540

7.2 ISO 21528-2:2004

Product description	Product format	Product code
VRBGA (ISO)	Dehydrated Culture Media (CM)	CM1082B - 500g
Oxidase Test	Kit/Reagent	R21540



Coliforms

Most probable number technique

Coliform bacteria are commonly used as bacterial indicators of sanitary quality in foods and water. They are Gram-negative rods, which can ferment lactose with the production of acid and gas when incubated at 35°C to 37°C. Coliforms can be found in the faeces of warm-blooded animals and while themselves are not causes of serious illness, are easy to culture, and their presence is used to indicate that other pathogenic organisms of faecal origin may be present.



Add 10mL of liquid sample or initial suspension to each of 3 tubes of 10mL of **Lauryl Sulphate Tryptose Broth** (CM0451)

Incubate for 24 hr ± 2 hr at 30°C or 37°C

AND

MPN

Add 1mL of liquid sample or initial suspension to each of 3 tubes of 10mL of

Lauryl Sulphate Tryptose Broth (CM0451)

Proceed in the same way with further dilutions

Incubate for 24 hr ± 2 hr at 30°C or 37°C If negative incubate for a further 24 hr

CONFIRMATION

Use a loopful from each tube to inoculate 10mL of **Brilliant Green Lactose Bile Broth (CM0031)**

Incubate for 24 hr ± 2 hr at 30°C or 37°C If negative incubate for a further 24 hr

AND

Use a loopful from each positive tube (gas and/or turbidity) to inoculate 10mL of **Brilliant Green Lactose Bile Broth (CM0031)**

> Incubate for 24 hr ± 2 hr at 30°C or 37°C If negative incubate for a further 24 hr

REPORT RESULTS

Calculate MPN



Coliforms

Most probable number technique

8 ISO 4831:2006

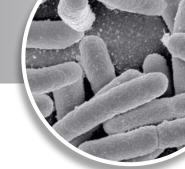
Product description	Product format	Product code
		CM0451B - 500g
Loury Culphoto Tryptogo Proth	Dehydrated Culture Media (CM)	CM0451R - 2.5Kg
Lauryl Sulphate Tryptose Broth		CM0451T – 5Kg
	Tube	TV5201G
Brilliant Green Bile Broth		CM0031B - 500g
	Dehydrated Culture Media (CM)	CM0031R - 2.5Kg
	Denyurated Culture Media (CM)	CM0031T - 5Kg
		CM0031K - 25Kg
	Tube	TV5009E
	Universal	B00345E
	Ulliversal	EB0345E



Coliforms

Colony-count technique

Coliform bacteria are commonly used as bacterial indicators of sanitary quality in foods and water. They are Gram-negative rods, which can ferment lactose with the production of acid and gas when incubated at 35°C to 37°C. Coliforms can be found in the faeces of warm-blooded animals and while themselves are not causes of serious illness, are easy to culture, and their presence is used to indicate that other pathogenic organisms of faecal origin may be present.



ISOLATION

1mL into sterile Petri dish (duplicate) + overlay VRBLA (ISO) Agar (CM0968)

Incubate for 24 hr \pm 2 hr at 30-37°C (as agreed)

CONFIRMATION

Brilliant Green Bile Broth (CM0031)

Incubate for 24 hr at 30°C or 37°C + durhams tube

Look for gas production

Coliforms

Colony-count technique

9 ISO 4832:2006

Product description	Product format	Product code
	Dehydrated Culture Media (CM)	CM0031B - 500g
		CM0031R - 2.5Kg
		CM0031T - 5Kg
Brilliant Green Bile Broth		CM0031K - 25Kg
	Tube	TV5009E
	Universal	B00345E
		EB0345E
VRBLA (ISO)	Dehydrated Culture Media (CM)	CM0968B - 500g



Clostridium perfringens

Colony-count technique

Of the Clostridia family *Clostridium perfringens* is the most commonly isolated from food. *C. perfringens* is a Gram-positive anaerobic sporulating bacillus unusual among Clostridia in being non-motile. Categorised into sub categories dependant upon the toxin produced, it is a key food poisoning pathogen in meat dishes.



ISOLATION

1mL into Petri dish Add SC agar at (44–47°C) Mix with rotation overlay 10mL Perfringens Agar

Incubate for 20 hr \pm 2 hr at 37°C in an anaerobic atmosphere

Inoculate colonies into

Fluid Thioglycollate Medium (CM0173)

Incubate for 18-24 hr at 37°C in an anaerobic atmosphere

If not well isolated streak onto SC Agar Inoculate FTM CM0173

CONFIRMATION

Inoculation of Lactose Sulphite Medium

Incubate for 18-24 hr at 46°C aerobic atmosphere in a water bath

Inoculation of Nitrate Motility Medium Lactose Gelatin Medium

Incubate for 24 hr at 37°C in an anaerobic atmosphere

REPORT RESULTS

Positive (C. perfringens)

Examine for typical reactions

Clostridium perfringens

Colony-count technique

10 ISO 7937:2004

Product description	Product format	Product code
		B00211Z – 80x39mL
		B01045M – 10x100mL
		B00157M – 10x100mL
		B00157Z – 10x450mL
		B00211M – 10x100mL
	Bottle	B00368F – 24x15mL
	Dottie	B00368M – 10x100mL
		B00368Y – 24x30mL
Fluid Thioglycolate Medium		B00510M – 10x100mL
Truid Trilogrycolate Medium		B00510V – 10x500mL
		B008760 – 10x80mL
		B00990R – 10x200mL
		CM0173B - 500g
	Dehydrated Culture Media (CM)	CM0173R – 2.5Kg
	Denyurated Culture Media (CM)	CM0173T – 5Kg
		CM0173K – 25Kg
	Tube	TV5001D
	Universal	B00211G

Part 1: Detection method

Campylobacter species

The Campylobacter genus is part of the family Campylobacteraceae and is a microaerophilic organism, Gram-negative, oxidase positive, spiral-shaped rods with flagella. Two key species within the genus are C. jejuni and C. coli, both a cause of the majority of diarrheal illness from poultry.



SELECTIVE ENRICHMENT

Add Xg to 9mL of Bolton Broth (CM0983 + SR0203/SR0183 + SR0048)

ENRICHMENT

Incubate in a microaerobic atmosphere for 4–6 hr at 37°C and then for 44 hr \pm 4 hr at 41.5°C

ISOLATION

mCCD Agar (CM0739 + SR0155) + 2nd medium, if preferred:

Karmali (CM0935 + SR0167)

Skirrow (CM0331 + SR0069)

Butzler (CM0331 + SR0214)

Incubate in a microaerobic atmosphere for 44 hr \pm 4 hr at 41.5°C

Characteristic colonies

CONFIRMATION

Columbia Blood Agar (CM0331 + SR0051)

Incubate for 24-48 hr at 41.5°C

IDENTIFICATION

Brucella Broth Growth at 25°C, 41.5°C (aerobic) Oxidase (MB0266A)

(CM0337 MHA + SR0051)

Part 2: Colony-count technique

Campylobacter species

The Campylobacter genus is part of the family Campylobacteraceae and is a microaerophilic organism, Gram-negative, oxidase positive, spiral-shaped rods with flagella. Two key species within the genus are *C. jejuni* and *C. coli*, both a cause of the majority of diarrheal illness from poultry.



ISOLATION

mCCDA (CM0739 + SR0155)

Incubate in a microaerobic atmosphere for 40-48 hr at 41.5°C

CONFIRMATION

Sub-culture to

Columbia Blood Agar (CM0331 + SR0051)

Incubate for 24–48 hr at 41.5°C

IDENTIFICATION

Brucella Broth Growth at 25°C, 41.5°C (aerobic)
Oxidase (MB0266A)

Campylobacter species

11.1 ISO 10272-1:2006

Product description	Product format	Product code
	Bottle	B01070S - 10x225mL
Bolton Broth	Debudrated Cultura Madia (CM)	CM0983B - 500g
	Dehydrated Culture Media (CM)	CM0983R - 2.5g
Bolton Broth Selective Supplement	Vial	SR0183E
Modified Bolton Broth Selective Supplement	Vial	SR0203E
Lysed Horse Blood	Vial	SR0048C
		CM0739B - 500g
mCCD Agar Base	Dehydrated Culture Media (CM)	CM0739R - 2.5Kg
		CM0739T – 5Kg
mCCDA Selective Supplement	Vial	SR0155E – 500mL
IIICCDA Selective Supplement	Viai	SR0155H – 2L
		P00966E – Bi-plate
mCCD Agar	Petri Dish	P05091A
		P00119A
		CM0337B - 500g
Muller Hinton Agar Base	Debudrated Cultura Madia (CM)	CM0337R - 2.5Kg
Wuller Hillon Agai base	Dehydrated Culture Media (CM)	CM0337T - 5Kg
		CM0337K - 25Kg
Defibrinated Sheep Blood	Vial	SR0051C
Muller Hinton Agar + Sheep Blood	Petri Dish	PB0431A
Wuller Hillon Agar + Sheep blood	reui disii	PB5007A
	Bottle	B00966M – 10x100mL
		CM0331B - 500g
Columbia Blood Agar Base	Debudrated Cultura Madia (CM)	CM0331R - 2.5Kg
	Dehydrated Culture Media (CM)	CM0331T - 5Kg
		CM0331K - 25Kg
		PB5008A
Columbia Blood Agar + Sheep Blood	Petri Dish	PB5039A
		PB0123A

Second Mediums of choice: ISO 10272-1:2006

Product description	Product format	Product code
Karmali Selective Medium Base	Dehydrated Culture Media (CM)	CM0935B - 500g
Karmali Selective Supplement	Vial	SR0167E
Karmali Selective Supplement (Modified)	Vial	SR0205E
Varmali Calaatiya Agar	Petri Dish	P05041A
Karmali Selective Agar	Petit disti	P05219E – Bi-plate
	Bottle	B00966M - 10x100mL
		CM0331B - 500g
Columbia Blood Agar Base	Debudrated Culture Media (CM)	CM0331R - 2.5Kg
	Dehydrated Culture Media (CM)	CM0331T - 5Kg
		CM0331K - 25Kg
Skirrow Selective Supplement	Vial	SR0069E
Columbia Agar - Skirrow	Petri Dish	PB0118A
Butzler Selective Supplement	Vial	SR0214E

Campylobacter species

11.2-3 ISO 10272-2:2006 (Includes Brilliance CampyCount as an ISO 16140 validated alternative to mCCDA)

Product description	Product format	Product code
		CM0739B - 500g
mCCD Agar Base	Dehydrated Culture Media (CM)	CM0739R - 2.5Kg
		CM0739T – 5Kg
mCCDA Selective Supplement	Vial	SR0155E – 500mL
Incode Selective Supplement	Viai	SR0155H – 2L
		P00966E – Bi-plate
mCCD Agar	Petri Dish	P05091A
		P00119A
Defibrinated Sheep Blood	Vial	SR0051C
	Bottle	B00966M – 10x100mL
		CM0331B - 500g
Columbia Blood Agar Base	Dehydrated Culture Media (CM)	CM0331R - 2.5Kg
	Derryurateu Guiture ivieula (Givi)	CM0331T - 5Kg
		CM0331K - 25Kg
Columbia Blood Agar + Sheep Blood		PB5008A
	Petri Dish	PB5039A
		PB0123A

11 Alternative method

Product description	Product format	Product code
Brilliance CampyCount Agar	Petri Dish	P01185A
O.B.I.S. Campy	Kit	ID0600M

11 Gas Generation—All above methods

Product description	Product format	Product code
		CN0025A - 2.5L Jar
CampyGen	For Microaerophilic Gas Conditions	CN0035A - 3.5L Jar
		CN0020C-1-4 plates
AnaeroJar	2.5L Capacity Jar	AG0025A
Anaerobic Jar	3.5L Capacity Jar	HP0011A

Brilliance CampyCount



OBSERVATION MADE SIMPLE

• Dark red colonies on a clear background

QUANTITATIVE

 Novel selectivity enables accurate, quantitative recovery of target organisms

ACCURATE CALCULATION

• Transparent medium allows enumeration on plate readers

EASY IDENTIFICATION

Reduced Campylobacter swarming for improved isolation of individual colonies

VALIDATED

ISO 16140 validated by MicroVal

ISO 16140 Validation

Brilliance CampyCount Agar has been validated and approved by MicroVal according to the ISO 16140:2003 Standard against the reference method ISO 10272-2: 2006 for the selective enumeration of thermotolerant *Campylobacter* spp., in particular *C. jejuni* and *C. coli*, in poultry products. For flexibility, this study included both the O.B.I.S. Campy kit and DrySpot Campylobacter latex tests as alternative confirmation methods to those described in the reference method ISO 10272-2: 2006. The MicroVal certificate is available in PDF format from www.microval.org.

Sensitivity was tested using a total of 81 Campylobacter strains isolated from poultry and associated environments and specificity was tested using 139 non-target strains.

Media	Specificity (n=139)	Sensitivity (n=81)
mCCDA	91%	100%
Brilliance CampyCount Agar	99%	100%

Protocol for enumeration of C. jejuni and C. coli using Brilliance CampyCount Agar



Day 0: Plating

Dilute sample in appropriate diluent

Plus

In duplicate, spread 0.1mL of appropriate dilution onto 2x *Brilliance* CampyCount Agar plates

Incubate for 48 hr \pm 1 hr at 41.5°C in a microaerobic atmosphere



Day 2: Results

If present, select at least 5 well isolated, dark red colonies



Confirm

Confirm using O.B.I.S. Campy

Alternatively, confirm colonies using standard ISO methods

Horizontal method for the enumeration of

presumptive Bacillus cereus

Most probable number technique and detection methods

A sporulating, Gram-positive organism that grows aerobically. Most commonly isolated from rice, cereals and pasta, its ability to cause food poisoning and spoil foods is well known. The ability of *Bacillus cereus* to produce two separate toxins that cause vomiting or diarrhea in relatively short incubation times is a more recent discovery, and has led to tighter controls around detection in certain foods.

SAMPLE PREPARATION

Xg into 9mL of appropriate diluent

10mL of initial suspension into 3 tubes of double-strength TSP Broth (CM0129 + SR0099)

Incubate for 48 hr ± 4 hr at 30°C

1mL of initial suspension into 3 tubes of TSPB single-strength or 1mL of each further dilution into 3 tubes of single-strength TSP Broth (CM0129 + SR0099)

Incubate for 48 hr \pm 4 hr at 30°C

ISOLATION

PEMBA Agar for 18–24 hr at 37° and additional 24 hr if necessary (CM0617 + SR0099 + SR0047)

OR

MYP Agar at 30°C for 18–24 hr and additional 24 hr if necessary (CM0929 + SR0099 + SR0047)

Selection of 3 suspect colonies

CONFIRMATION

Colonies isolated from PEMBA:

· Haemolysis test or microscopic examination

Colonies isolated from MYP:

- · Haemolysis test
- Tryptone Soya Agar

s/s = single strength d/s = double strength XmL = sample size Xg = sample size

Calculate MPN

presumptive Bacillus cereus

Most probable number technique and detection methods

12 ISO 21871:2006

Product description	Product format	Product code
		CM0129B - 500g
TSP Broth Base	Debudrated Culture Media (CM)	CM0129R - 2.5Kg
13r blutt base	Dehydrated Culture Media (CM)	CM0129T – 5Kg
		CM0129K – 25Kg
	Bottle	B01032J – 10x90mL
MVD Ager Page	Dehydrated Culture Media (CM)	CM0929B - 500g
MYP Agar Base	Petri Dish	P05133A
	retti bisii	P00711A
Polymixin B Supplement	Vial	SR0099E
Egg Yolk Supplement	Bottle	SR0047C - 100mL
		CM0617B - 500g
Parillus caraus Calactina Area (PEMPA)	Dehydrated Culture Media (CM)	CM0617R - 2.5Kg
Bacillus cereus Selective Agar (PEMBA)		CM0617T – 5Kg
	Petri Dish	P05048A
Defibrinated Sheep Blood	Vial	SR0051C
	Bottle	B00965Z – 10x450mL
Blood Agar No. 2 + Sheep Blood	Dottie	B00965M – 10x100mL
	Petri Dish	PB0115A

Horizontal method for the enumeration of

presumptive Bacillus cereus

Colony-count technique at 30°C

A sporulating, Gram-positive organism that grows aerobically. Most commonly isolated from rice, cereals and pasta, its ability to cause food poisoning and spoil foods is well known. The ability of *Bacillus cereus* to produce two separate toxins that cause vomiting or diarrhea in relatively short incubation times is a more recent discovery, and has led to tighter controls around detection in certain foods.



SAMPLE PREPARATION

 $X_{\rm g}$ in 9mL of diluent according to sample type

ISOLATION

Surface inoculation onto

MYP Agar (CM0929 + SR0099 + SR0047)

(0.10mL) to 2 plates

Or 1.0mL to 3 plates (in duplicate)

Incubate for 18–24 hr at 30°C (and an additional 24 hr if colonies are not clearly visible) Streak or stab selected colonies onto Sheep Blood Agar

CONFIRMATION

Haemolysis reaction using Sheep Blood Agar

Incubate for 24 hr ± 2 hr at 30°C

Positive is presumptive for Bacillus cereus

presumptive Bacillus cereus

Colony-count technique at 30°C

13 ISO 7932:2004

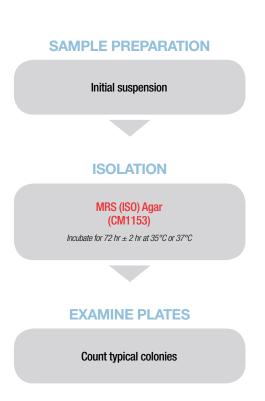
Product description	Product format	Product code
MYP Agar Base	Dehydrated Culture Media (CM)	CM0929B - 500g
Polymixin B Supplement	Vial	SR099E
Egg Yolk Supplement	Bottle	SR0047C - 100mL
Defibrinated Sheep Blood	Vial	SR0051C
	Bottle	B00965Z – 10x450mL
Blood Agar Base No. 2 + Sheep Blood		B00965M - 10x100mL
	Petri Dish	PB0115A

Horizontal method for the enumeration of

mesophilic lactic acid bacteria

Colony-count technique at 30°C

Lactic acid bacteria are Gram-positive, acid tolerant, rods or cocci that usually produce lactic acid as the major metabolic end product of carbohydrate fermentation. The industrial importance of *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus*, and *Streptococcus* species in brewing, baking and food preparation is well known, but their presence in other foods can cause spoilage, unwanted characteristics or appearance, and in some cases, mild food poisoning.





mesophilic lactic acid bacteria

Colony-count technique at 30°C

14 ISO 15214:1998

Product description	Product format	Product code
MDC (ICO) Agor	Dehydrated Culture Media (CM)	CM1153B - 500g
MRS (ISO) Agar	Petri Dish	P01228A

14 Gas Generation—All above methods

Product description	Product format	Product code
		CN0025A - 2.5L Jar
CampyGen	For Microaerophilic Gas Conditions	CN0035A - 3.5L Jar
		CN0020C - 1-4 plates
AnaeroJar	2.5L Capacity Jar	AG0025A
Anaerobic Jar	3.5L Capacity Jar	HP0011A

Horizontal method for detection of

Shigella species

Shigella are related to *E. coli* phenotypically and genetically appear the same. All species of Shigella are pathogenic to humans and cause dysentery. The increasing awareness of Shigella as a foodborne pathogen has lead to many advances in detection and increased regulation around detection.



SELECTIVE ENRICHMENT

9Xg or 9XmL in Shigella Broth + 0.5 μg novobiocin Homogenise and adjust to pH7.0 \pm 0.2

ENRICHMENT

Incubate in an anaerobic atmosphere for 16 hr \pm 4 hr at 41.5°C

ISOLATION

MacConkey Agar (CM0115) XLD Agar (CM0469) Hektoen Enteric Agar (CM419)

Incubate for 20 hr \pm 4 hr at 37°C \pm 1°C

Characteristic colonies onto Nutrient Agar

Incubate for 20 hr \pm 4 hr at 37°C \pm 1°C

CONFIRMATION

TSI Agar (CM0277) UREA Agar (CM0053)

L-Lysine Decarboxylation Medium

Shigella species

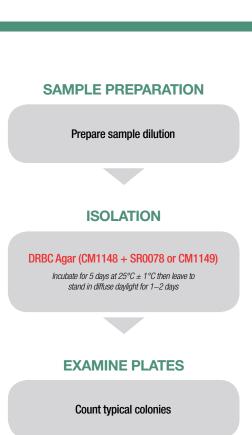
15 ISO: 21567:2004

Product description	Product format	Product code
		CM0115B - 500g
	Dehydrated Culture Media (CM)	CM0115R - 2.5Kg
MacConkey Agar N°3	Deriyurated Culture Media (CM)	CM0115T – 5Kg
Wacconkey Agai N 3		CM0115K – 25Kg
	Petri Dish	P05002A
	רפנוז טואוו	P00495A
		CM0469B - 500g
	Dehydrated Culture Media (CM)	CM0469R - 2.5Kg
XLD Agar		CM0469T – 5Kg
	Petri Dish	P00164A
	ו פנוז טוטוו	P05057A
		CM0419B - 500g
	Dehydrated Culture Media (CM)	CM0419R - 2.5Kg
Hektoen Enteric Agar	Deriyurated Culture Media (Civi)	CM0419T – 5Kg
Herioen Enteric Agai		CM0419K – 25Kg
	Petri Dish	P05100A
	רפנוז טואוו	P00142A
Triple Sugar Iron Agar (TSI)	Dehydrated Culture Media (CM)	CM0277B - 500g
Tiple Juyai IIUII Ayai (131)	Tube	TV5074D
	Dehydrated Culture Media (CM)	CM0053B - 500g
Urea Agar	Slope	B00337B – 24x3mL
	Siope	EB0337B – 200x3mL

Part 1: Colony-count technique in products with water activity greater than 0.95

Yeasts and moulds

Yeast and mould are widespread in nature and grow especially well in organic environments. Yeasts appear as single, separate, oval cells when mature, whereas moulds tend to link together to form long, branding hyphae. Some yeast and mould may produce toxic metabolites known as mycotoxins. Most mycotoxins are resistant to destruction by food processing or cooking. Food types particularly prone to yeast and mould infection include grains, nuts, beans and fruits.

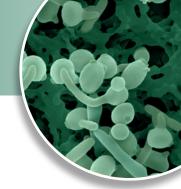




Part 2: Colony-count technique in products with water activity less than or equal to 0.95

Yeasts and moulds

Yeast and mould are widespread in nature and grow especially well in organic environments. Yeasts appear as single, separate, oval cells when mature, whereas moulds tend to link together to form long, branding hyphae. Some yeast and mould may produce toxic metabolites known as mycotoxins. Most mycotoxins are resistant to destruction by food processing or cooking. Food types particularly prone to yeast and mould infection include grains, nuts, beans and fruits.



SAMPLE PREPARATION

Prepare sample dilution

ISOLATION

0.1mL of test sample onto
DG18 Agar (CM1150 + SR0078 or CM1151)
Onto a second plate transfer 0.1mL
of the first decimal dilution onto
DG18 Agar (CM1150 + SR0078 or CM1151)

Incubate for 5–7 days at 25°C ± 1°C then leave to stand in diffuse daylight for 1–2 days

EXAMINE PLATES

Count typical colonies

Yeasts and moulds

16.1 ISO 21527-1:2008

Product description	Product format	Product code
DRBC (ISO) Agar Base	Dehydrated Culture Media (CM)	CM1148B - 500g
Chloramphenicol Supplement	Vial	SR0078E
		SR0078H
Pre-supplemented DRBC (ISO) Agar	Dehydrated Culture Media (CM)	CM1149B - 500g
	Petri Dish	P01227A

16.2 ISO 21527-2:2008

Product description	Product format	Product code
DG18 (ISO) Agar Base	Dehydrated Culture Media (CM)	CM1150B - 500g
Chloramphenicol Supplement	Vial	SR0078E
		SR0078H
Pre-supplemented DG18 (ISO) Agar	Dehydrated Culture Media (CM)	CM1151B - 500g

Precautions

ISO Standard Formulation Conformity

The Thermo Scientific products featured in this brochure conform to the stated ISO standard formulation in their relevant organism section.



Products Not Included

The Thermo Scientific product range may include a dehydrated or prepared culture media with the same or similar name to the media described within an ISO standard, but these products do not appear in this brochure because they do not conform to the formulation set out in the ISO standard.

'Specials'

Some products listed in this brochure are classified as 'special' formulations or formats and are not held in stock, therefore they may be subject to extended lead times for ordering.

Ancillary Products

The Thermo Scientific product range contains many products that provide solutions to anaerobic or microaerophilic cultivation, incubation and refrigeration that can be used in conjunction with the culture media offering. Where applicable these products have been identified underneath the culture media offering. These products are not recommended by ISO but can be used along with the culture media to complete the testing methodology.

Alternative Products

In some sections of this brochure there are products or methods that have been added as validated alternatives to the stated method. These alternatives have been validated by either NF Validation or another validation body using the ISO 16140 Standard against the stated method, and have been given AFNOR or MicroVal certification.

ISO Flow Diagrams

The ISO flow diagrams used in this brochure have been simplified for easy identification of available products. To follow the ISO testing protocol and see the full flow description please refer to the specified ISO standard document.

