Scharlau

The wise choice

Food Microbiology

Standardised procedures
for the microbiological analysis of foodstuffs
in accordance with ISO standards

Scharlab has accumulated over 35 years of experience in the production of microbiology culture media. In 1991, we also started to sell ready-to-use culture media to satisfy the market's changing preference for such products instead of dehydrated culture media.

Scharlau's portfolio of culture media includes media specifically created for quality control in different sectors:

- Pharmaceutical and veterinary industries
- Cosmetics
- Food and beverage sector
- Water analysis and environmental laboratories
- Hospitals and clinics

All of our dehydrated and ready-to-use culture media are produced in accordance with our own internal Quality Standards and Quality Management Practices, as set out in ISO 9001:2015, while also fulfilling the requirements of the CE brand.

Our culture media also comply with validated methods, International Standards and other microbiological regulations such as ISO, Harmonised Pharmacopoeia Methods, European Pharmacopoeia, USP, FDA, etc.

In relation to the food and beverage sector, we offer a wide selection of culture media formulated and controlled according to the corresponding directives and requirements:

- Broths and diluents for sample preparation
- Selective and non-selective culture media
- Culture media for confirmation tests

Introduction

The COMMISSION REGULATION (EC) No. 1441/2007 amending REGULATION (EC) No. 2073/2005 of 5 December 2007 on microbiological criteria for foodstuffs establishes:

I. The microbiological criteria for certain microorganisms and the rules food business operators must follow when implementing the general and specific hygiene measures set out in Article 4 of Regulation (EC) No 852/2004.

II. That food business operators must guarantee that foodstuffs comply with the relevant microbiological criteria set out in Annex I of Regulation (EC) No. 2073/2005.

Furthermore, Regulation (EC) No. 2073/2005 stipulates the microbiological criteria for foodstuffs, food safety and the process hygiene criteria for meat and products thereof, milk and dairy products, egg products, fishery products, and vegetables, fruits and products thereof. These microbiological criteria are determined using ISO reference analytical methods.

This document intends to provide a clear and understandable summary of the ISO reference analytical methods included in COMMISSION REGULATION (EC) No. 1441/2007 of 5 December 2007. It also includes a summary of COMMISSION REGULATION (EU) 2017/1495 amending REGULATION (EC) No. 2073/2005 of 23 August 2017 as regards *Campylobacter* in broiler carcasses. The featured methods are:

- **ISO 4833: Microbiology of the food chain.** Horizontal method for the enumeration of microorganisms.
- **ISO 6579: Microbiology of the food chain.** Horizontal method for the detection of *Salmonella spp.*
- **ISO 6888-1/2: Microbiology of food and animal feeding stuffs.** Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium. Part 2: Technique using rabbit plasma fibrinogen agar medium.
- **ISO 7932: Microbiology of food and animal feeding stuffs.** Horizontal method for the enumeration of presumptive *Bacillus cereus.* Colony-count technique at 30°C.
- **ISO 10272-2: Microbiology of the food chain.** Horizontal method for detection and enumeration of *Campylobacter spp.* Part 2: Colony-count technique.
- **ISO 11290-1/2: Microbiology of the food chain.** Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of ***Listeria spp.*** Part 1: Detection method. Part 2: Enumeration method.
- **ISO 16649-1/2/3: Microbiology of the food chain.** Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli.* Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl ß-D-glucuronide. Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl ß-D-glucuronide. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide.
- **ISO 21528-1/2: Microbiology of the food chain.** Horizontal method for the detection and enumeration of *Enterobacteriaceae.* Part 1: Detection of *Enterobacteriaceae.* Part 2: Colony-count technique.
- **ISO 22964: Microbiology of the food chain.** Horizontal method for the detection of *Cronobacter spp.*

Illustrative symbols for the different culture media packaging formats

Boxes with five sachets of dehydrated media (each sachet prepares 500 ml of media)		20 tubes of 10 ml / 9 ml (broth media)	
500 g		20 tubes of 15 ml / 10 ml (agar media)	
20 plates of 90 mm	90	20 tubes of slant tubes	
10 flasks of 100 ml / 200 ml / 225 ml (broth) 1 flask of 100 ml and 1 litre (additive)		Bag of 2 litres / 3 litres / 5 litres (Flexibags)	
		Lyophilised supplements LYO1 (10 vials)	LYO
10 frascos de 100 ml / 200 ml (agar media)		Flask with screw cap and dropper	

NOTE: For dehydrated media presented in 500 g packs ( ), we can offer other formats, including 5 and 25 kg packs. For more information write to: helpdesk@scharlab.com.

Total enumeration • ISO 4833-1

Microbiology of the food chain.

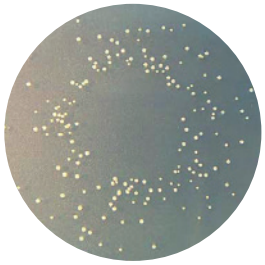
Horizontal method for the enumeration of microorganisms.

Part 1: **Colony count at 30°C by the pour plate method.**

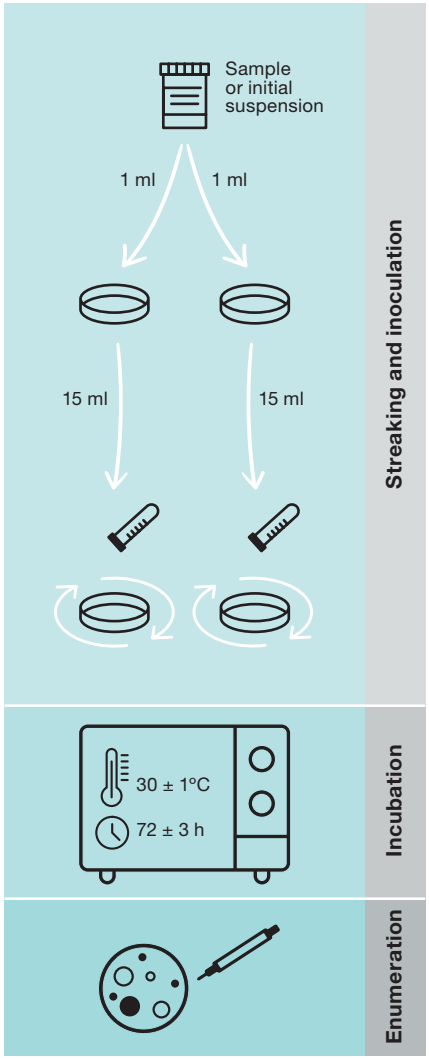
This part of ISO 4833 describes a horizontal method for the enumeration of microorganisms that are able to grow and form colonies in a solid media after aerobic incubation at 30°C.

Method applicable to:

- Cattle carcasses.
- Sheep carcasses.
- Goat carcasses.
- Horse carcasses.
- Pig carcasses.
- Minced meat.
- Mechanically separated meat.



PCA
Staphylococcus aureus ATCC 6538



PROCEDURE

Streaking and inoculation:

- Transfer 1 ml of the test sample, if liquid, or 1 ml of the initial suspension (10^{-1} dilution) to two sterile, empty Petri dishes.
- Transfer 1 ml of the 10^{-1} dilution (if the product is liquid) or 1 ml of the 10^{-2} dilution (for other types of product).
- Repeat the process for the other dilutions.
- Pour 12-15 ml of PCA (Plate Count Agar) into each dish.

NOTE: The agar must be at a temperature range of 44-47°C.

- Carefully mix the medium and sample by rotating the Petri dishes, then allow them cool.
- Once cooled and as an option, they can be covered with 4 ml of melted agar (44-47°C), then allow to cool.

Incubation:

- Invert the prepared plates and incubate them at 30°C ± 1°C for 72 h ± 3 hours.

Enumeration:

- Count the CFU in the plates that contain less than 300 colonies.

			90		
Plate Count Agar (PCA)	01-161-500	01-161BA05	064-PA0024	064-BA1005	064-TA0325
			064-PA0144 [1]	064-BA3038	
			064PA0024I		
Plate Count Modified Agar	01-329-500 [2]	01-329BA05			
Plate Count Skim Milk Agar	01-412-500	01-412BA05		064-BA2120	
				064-BA3057	

[1] With TTC [2] Recommended for the pour plate method

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ISO 6579 • *Salmonella* spp.

This international method specifies a horizontal method for the detection of *Salmonella,* including *Salmonella typhi* and *Salmonella paratyphi,* and can be applied to:

Method applicable to:

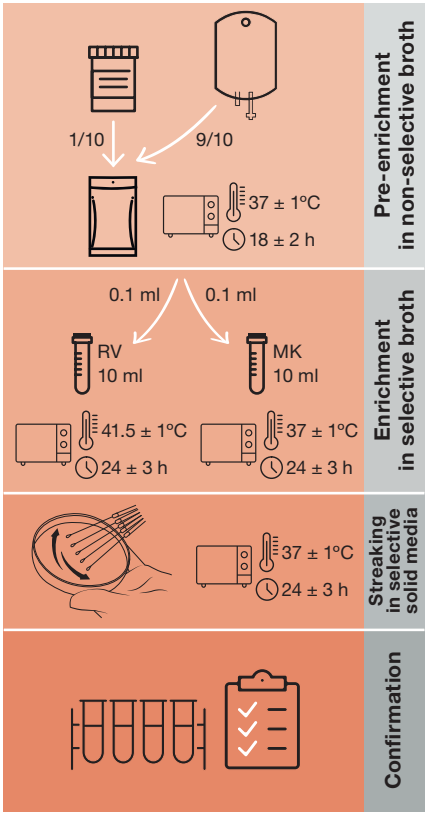
- Minced meat and meat preparations intended to be eaten raw.
- Minced meat and meat preparations made from poultry meat intended to be eaten cooked.
- Gelatine and collagen.
- Ice-cream.
- Egg products and foodstuffs containing raw egg.
- Cooked crustaceans and molluscan shellfish.
- Live bivalve molluscs, echinoderms, tunicates and marine gastropods.
- Sprouted seeds.
- Ready-to-eat pre-cut fruits and vegetables.
- Unpasteurised fruit juices.
- Dried infant formula and dried dietary foods intended for infants below 6 months.
- Pig carcases.
- Chicken and turkey broiler carcasses.
- Environmental samples from food production and manipulation areas.

Microbiology of food and animal feeding stuffs.

Horizontal method for the detection of *Salmonella* spp.

XLD
Salmonella typhimurium ATCC 14028

PROCEDURE



Pre-enrichment in non-selective broth:

- Inoculate 25 g or 25 ml of the sample in 225 ml of Peptone Water at room temperature (1/10 dilution).
- Incubate at 37°C ± 1°C for 18 h ± 2 hours.

Enrichment in selective broth:

- Inoculate a tube of 10 ml of Rappaport-Vassiliadis broth with 0.1 ml from the pre-enrichment broth and incubate at 41.5°C ± 1°C for 24 h ± 3 hours. At the same time, streak a plate of MSRV agar with the same amount of pre-enrichment broth.
- Inoculate a tube of 10 ml of Müller-Kauffmann Tetrathionate Broth Base with Novobiocin with 1 ml from the pre-enrichment broth and incubate at 37°C ± 1°C for 24 h ± 3 hours.

Streaking in selective solid media:

- Streak an XLD agar plate and incubate at 37°C ± 1°C for 24 h ± 3 hours and a second choice agar such as Brilliant Green Agar, Hektoen Enteric Agar, Salmonella Shigella Agar or Salmonella Plus Chromogenic Agar.

Confirmation:

- Re-streak the presumptive colonies in nutrient agar at 34-38°C for 24 h ± 3 hours. Confirm the colonies using serological and biochemical tests.
  - TSI Agar: 37°C for 24 h ± 3 h
  - Urea Agar: 37°C up to 24 h
  - Decarboxylase Lysine Broth: 37°C for 24 h ± 3 h
  - ß-galactosidase detection: 37°C up to 24 h
  - Indole Test: 37°C for 24 h ± 3 h

			90				10V
Brilliant Green Agar (BGA)	01-203-500	01-203BA05	064-PA0045				
Buffered Peptone Water (ISO)	02-277-500	02-277BA05		064-BA4010	064-TA0100	064-BA01-2	
				064-BA0486		064-BA01-3	
				064-BA6000		064-BA01-5	
				064-BA6011			
				064-BA6012			
				064-BA8043			
CHROMagar™ Salmonella Plus	00000SA162		064-PA0083				
Decarboxylase Lysine Broth (Taylor)	02-336-500				064TA0125L		
Hektoen Enteric Agar	01-216-500	01-216BA05	064-PA0014				
Indole test					064-TA0132		
Modified Semi-solid Rappaport-Vassiliadis (MSRV) Medium Base	03-376-500	03-376BA05					06-139LYO1
Müller-Kauffmann Tetrathionate Broth Base	02-335-500	02-335BA05		064-BA1024	064-TA1024		06-017LYO1
				064-V11108[1]			
Nutrient Agar (APHA, ISO)	01-144-500	01-144BA05					
ONPG-FDA-MUG-INDOL Test					064-TA0203		
Rappaport-Vassiliadis Broth	02-379-500	02-379BA05			064-TA0198		
Salmonella Shigella Agar (SS Agar)	01-555-500	01-555BA05	064-PA0028				
Triple Sugar Iron Agar (TSI Agar)	01-192-500	01-192BA05			064-TA0177		
Urea Agar Base	01-261-500	01-261BA05		06-083-100[1]	064-TA0148		
Xylose Lysine Deoxycholate Modified Agar (XLD Modified Agar)	01-552-500	01-552BA05	064-PA1035				

[1] It is an additive

Coagulase-Positive Staphylococci • ISO 6888-1/2

Microbiology of food and animal feeding stuffs.

Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species).

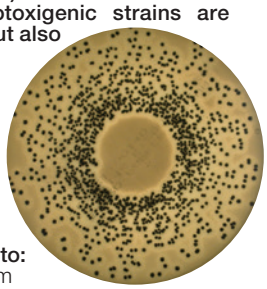
Part 1: **Technique using Baird-Parker agar medium.**

Part 2: **Technique using rabbit plasma fibrinogen agar medium (RPF).**

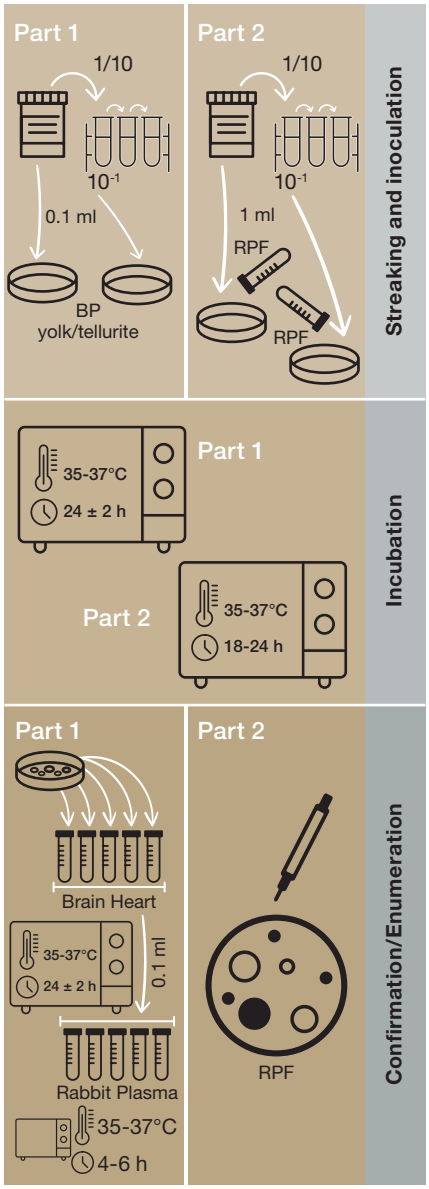
ISO 6888 describes two horizontal methods (Part 1 and Part 2) for the enumeration of coagulase-positive staphylococcus among which enterotoxigenic strains are encountered. Mainly concerned with *Staphylococcus aureus,* but also *Staphylococcus intermedius* and *Staphylococcus hyicus.*

In general, Part 1 and Part 2 are applicable to:
- Cheeses made with pasteurised milk or treated with lower heat treatment.
- Ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment.
- Unripened soft cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment.
- Milk powder and whey powder.
- Shelled and shucked products of cooked crustaceans and molluscan shellfish.

Part 2 only applies to:
- Cheeses made from raw milk.



Baird Parker
Staphylococcus aureus ATCC 25923



PROCEDURE

Streaking and inoculation:

Part 1:
- Transfer 0.1 ml of the sample, if liquid, or 1 ml of the initial suspension (10^{-1} dilution) to two Baird-Parker plates with sterile Egg Yolk Tellurite Emulsion.

Part 2:
- Transfer 1 ml of the sample, if liquid, or 1 ml of the initial suspension (10^{-1} dilution) to two sterile, empty Petri dishes.
- Transfer 1 ml to two other empty Petri dishes from the first decimal dilution.
- Repeat the process for the other dilutions.
- Add RPF agar to each Petri dish up to a depth of 3 mm.

Incubation:

Part 1:
- Incubate at 35°C or 37°C for 24 h ± 2 hours.
- Then incubate for another 24 h ± 2 hours.

Part 2:
- Mix the agars and incubate at 35 or 37°C for 18 to 24 hours.

NOTE (applicable to part 1 and 2): if necessary, incubate for a further 18 to 24 hours.

Confirmation/Enumeration:

Part 1:
- Inoculate five colonies in tubes of Brain Heart Infusion Broth and incubate at 35 or 37°C for 24 h ± 2 hours.
- Add 0.1 ml from each tube to 0.3 ml of Rabbit Plasma. Incubate at 35 or 37°C for 4 to 6 hours.
- The result is positive if the volume of the clot is more than half that of the original liquid.

Part 2:
- Count colonies which are small, convex, black or grey (can even be white) with a halo of precipitation.

Baird Parker Agar Base	01-030-500	01-030BA05	064-PA0008	064-BA1033		
				064-BA1018[1]		
Baird Parker RPF Agar	01-030-500		064-PA0145	064-BA1028		064-TA0155
				064-BA7038		
Brain Heart Infusion Broth	02-599-500	02-599BA05		064-BA6079	064-TA0143	
Coagulase Test (Rabbit Plasma)					064-PLA-CO	

[1] It is an additive

ISO 7932 describes a horizontal method for the enumeration of the viable presumptive *Bacillus cereus* by means of the colony-count technique at 30°C.

Method applicable to:

- Dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age.

MYP Agar
Bacillus cereus ATCC 11778

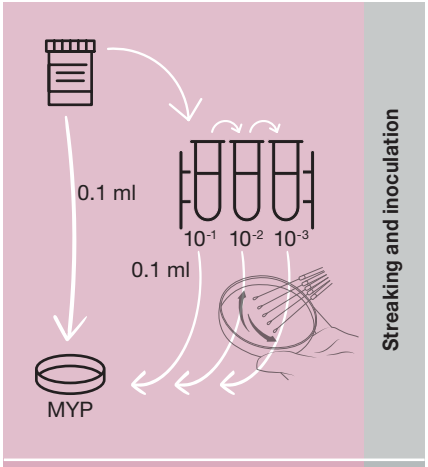
Microbiology of food and animal feeding stuffs.

Horizontal method for the enumeration of presumptive *Bacillus cereus*. Colony-count technique at 30°C.

ISO 7932 • *Bacillus cereus*

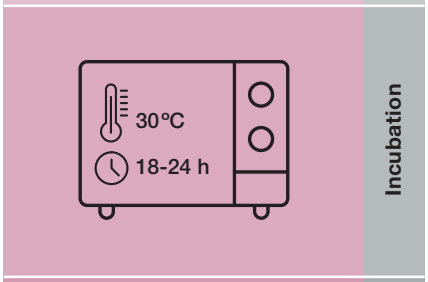
PROCEDURE

Make an initial suspension and its dilutions (1:10) for streaking.



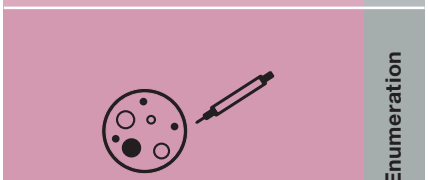
Streaking and inoculation:

- Transfer 0.1 ml of the sample, if liquid, or 0.1 ml of the initial suspension to two MYP agar plates.
- If there is an estimation of a low CFU, transfer 1 ml of the sample, if liquid, or 1 ml of the initial suspension to two MYP agar plates. Carry out in triplicate.
- Spread the sample and leave it to stand at room temperature for 15 minutes.



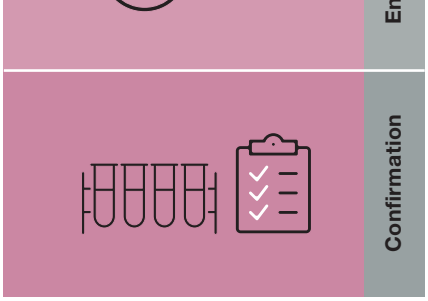
Incubation:

- Incubate at 30°C for 18 to 24 hours. If necessary, incubate for a further 24 hours.



Enumeration:

- Count the plates that contain less than 150 colonies (big, pink and with a halo of precipitation).



Confirmation:

- Streak five presumptive colonies in Blood Agar Base No. 2.
- Incubate at 30°C for 24 h ± 2 hours.
- Apply the haemolysis test to the presumptive colonies (*Bacillus cereus* will be positive in the test).

Bacillus cereus agar (MYP agar)	01-262-500	01-262BA05	064-PA0032	06-016-100[1] 064-BA2129	06-021LYO1
Blood Agar Base No. 2.	01-505-500	01-505BA05	064-PA9997		

[1] It is an additive

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Scharlau's plastic consumable material for the application of ISO standards

Threaded containers for samples

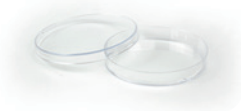
PP urine containers with PE cap. Writing surface on the side. Wide thermal and chemical resistance. The sterile models have been irradiated and are supplied in an individual bag. Two capacities available. The code PUC1501000, is delivered with the cap without threading.

Description	Cap. (ml)	Ext. dim. Ø x H (mm)	Sterile	Pack (u.)	Art. No.
With assembled cap	60	38x65	No	600	PSC0603000
Sterile with assembled cap	60	38x65	Yes	400	PSC0603001
With separate cap	150	58x72	No	500	PUC1501000
Sterile with assembled cap	150	58x72	Yes	250	PUC1501001

Sterile bags for homogenisation

Scharlau sterile bags for homogenisation processes and analysis of solid samples. Notably resistant to the stress processes they are subjected to in the homogenisers. Compatible with any homogeniser. Three models available. Bags without filter, bags with lateral filter for the separation of fibrous samples and bags with total filter for pasty samples. The filters allow filtration of the sample during the homogenisation process and separate the bag into two spaces: one to introduce the sample and another to facilitate sampling with the pipette.

Description	Cap. (ml)	Usable vol. (ml)	Porus size (µm)	Dim. W x H (mm)	Pack (u.)	Art. No.
Without filter	400	50-300	-	175x300	500 (20x25)	BAG0400-01
With lateral filter	400	50-300	< 250	190x300	500 (20x25)	BAG0400-02
With total filter	400	50-300	280	190x300	500 (20x25)	BAG0400-03



Petri plates

Scharlau Petri dishes are manufactured in high transparency PS following a fully automated process with strict quality controls, giving them the strength and transparency ideal for microbiological applications. Their great mechanical resistance makes them non-deformable. The dishes with winds facilitate the circulation of the air and avoid possible condensation. Aseptically produced in "Cleanroom ISO 6" rooms, a level of sterility is guaranteed. In addition, dishes are sterilised by radiation. They have a ring to facilitate and improve stability once stacked.

Descripción	Ø (mm)	Height (mm)	Amount/Bag	Pack (u.)	Art. No.
Aseptic ISO 6, 3 vents	90	14.2	20	480	PPD-90143A
Sterile, 3 vents	90	14.2	20	480	PPD-90143E

Filter tips

Compatible with the most common brands of pipettes. RNase & DNase free. Pyrogen free. Autoclavable at 121°C/15min. Graduation marks. High grade of transparency.

Vol. (µl)	Colour	Presentation	Pack (u.)	Art. No.
1 - 200	Natural	Bag	1.000	00PF1200-1
1 - 200	Natural	Rack	10x96	00PF1200-2
100 - 1000	Natural	Bag	1.000	PF10010001
100 - 1000	Natural	Rack	8x96	PF001000-2

Cylindrical flasks (Duchess type)

Duchess type flasks, cylindrical with wide neck, translucent white, made of high density polyethylene. For the collection, conservation and storage of samples. With black screw cap and translucent pressure shutter to ensure airtightness and product conservation.

Cap. (ml)	Mouth Ø (mm)	Ø x H (mm)	Pack (u.)	Art. No.
60	46	57x45	500	DUQ0000060
100	46	59x60	350	DUQ0000100
125	46	59x70	300	DUQ0000125
250	51	67x98	280	DUQ0000250
500	62	80x112	160	DUQ0000500
1000	86	102x151	90	DUQ0001000
2000	86	118x227	64	DUQ0002000

Conical tubes polypropylene

PP centrifuge tubes conical bottom. Leakproof closure. Easy-to-read graduation. CE marking. Cap also made of PP. commonly used in centrifugation, sample storage and molecular biology among other applications.

Volumen	Ext. Ø x H (mm)	Centrif. resist (g)	Sterile	Pack (u.)	Art. No.
15 ml	21x120	-	No	1000	PCT0151000
15 ml	21x120	-	Yes	1x1000	PCT0151001
50 ml	28x118	12000	No	500	PCT0501000
50 ml	28x118	12000	Yes	1x500	PCT0501002
50 ml with shirttail	28x118	12000	No	500	PCT0502000
50 ml with shirttail	28x118	12000	Yes	1x500	PCT0502002

Sterile disposable inoculating loops

Inoculation loops sterilised by radiation, manufactured in PS of 1 and 10 µl. Bags in peel-packs of 20 units with lot number and expiry date printed. Delivered with certified calibration and sterilisation.

Volumen (µl)	Colour	Presentation	Pack (u.)	Art. No.
1	Green	Bags 20 units	1000	PIL0030120
10	Blue	Bags 20 units	1000	PIL0041020

Universal tips for automatic pipettes

New range of Scharlau Polypropylene tips. Compatible with most brands of automatic pipettes (Sartorius, Brand, Eppendorf, Finnpipette, Gilson, Socorex, etc.). Available in bags, autoclavable racks, radiation sterilised racks and individually packed sterile tips.

Vol. (µl)	Colour	Presentation	Pack (u.)	Art. No.
5-200	Yellow	Bag	1000	000P5202-1
5-200	Yellow	Rack	10x96	000P5200-2
100-1000	Blue	Bag	1000	000P1000-1
100-1000	Blue	Rack	8x60	000P1000-2

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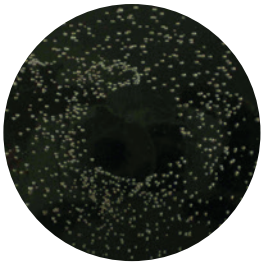
Part of ISO 10272 describes a horizontal method for the detection and enumeration of *Campylobacter spp.* in chicken broiler carcasses.

Method applicable to:

- Chicken broiler carcasses.



CCDA
Campylobacter jejuni ATCC 29428

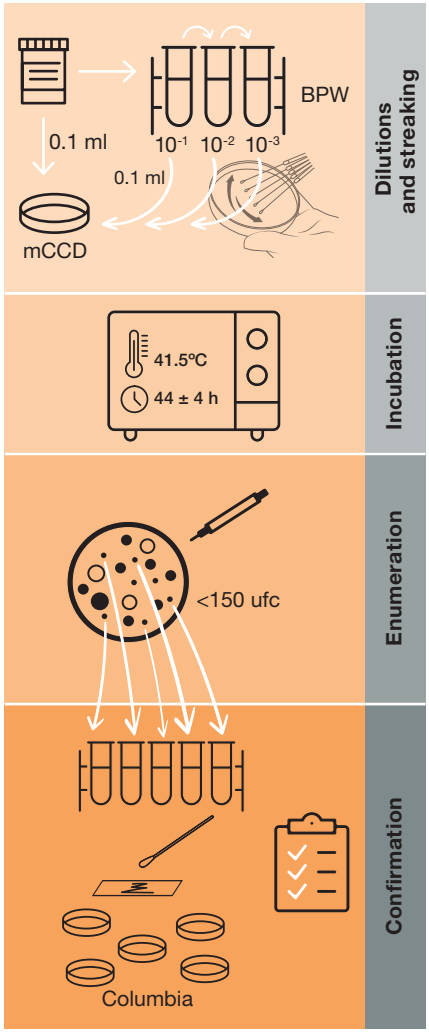
Microbiology of the food chain.

Horizontal method for detection and enumeration of *Campylobacter spp.*

Part 2: **Colony-count technique.**

ISO 10272-2 • *Campylobacter spp.*

PROCEDURE



Dilutions and streaking:

- From the sample, prepare serial dilutions in BPW.
- Streak 0.1 ml of the initial suspension, and the serial dilutions if necessary, in Modified CCD Agar (Charcoal Cefoperazone Deoxycholate).

Incubation:

- Incubate in a microaerophilic atmosphere at 41.5°C for 44 h ± 4 hours.

Enumeration:

- The typical colonies of *Campylobacter spp.* are greyish and often have a metallic sheen, and are flat and moist, with a tendency to spread.

  NOTE: The ability to identify *Campylobacter spp.* colonies is mostly learnt through experience as their appearance can change, not just between strains, but also between different batches of the same medium.
- Select the plates with less than 150 typical colonies and choose 5 from those for confirmation.

Campylobacter spp. confirmation:

- Streak the typical colonies in Columbia agar (Non-Selective Blood Agar).
- Incubate in a microaerophilic atmosphere at 41.5°C for 24 h-48 hours.
- Confirmation tests:
  - Morphology and motility: small curved bacilli.
  - Aerobic growth at 25°C: plate in Columbia agar and incubate at 25°C for 44 h ± 4 hours. No growth observed.
  - Oxidase test: positive.
  - Catalase test (optional): positive.

									Swabs
Blood Agar Base (Columbia)		01-034-500	01-034BA05	064-PA0004					
Buffered Peptone Water (ISO)		02-277-500	02-277BA05		064-BA4010	064-TA0100	064-BA01-2		
					064-BA0486		064-BA01-3		
					064-BA6000		064-BA01-5		
					064-BA6011				
					064-BA6012				
					064-BA8043				
Catalase Test Reagent					HI01351000				
					064-CL0234				
Modified CCD Agar (Charcoal Cefoperazone Deoxycholate)		01-685-500		064-PA0009				06-133LYO1	
Oxidase Test									06-120-050
	RE00650005								

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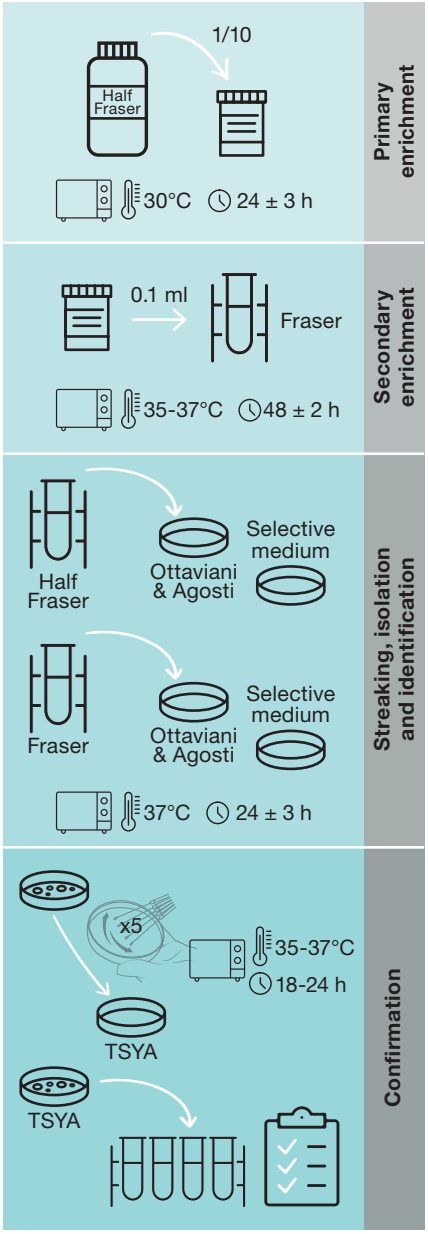
Listeria monocytogenes • ISO 11290-1

Microbiology of the food chain.
Horizontal method for the detection and enumeration of *Listeria monocytogenes.*
Part 1: **Detection method.**
Part 2: Enumeration method.

ISO 11290-1 describes a horizontal method for the detection of *Listeria monocytogenes,* a pathogen that can be found in food.

Method applicable to:

- Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes.
- Ready-to-eat foods able to support the growth of *Listeria monocytogenes,* other than those above.



PROCEDURE

Primary enrichment (preparation of the initial suspension):

- Add 25 g or ml of the sample to 225 g or ml of the primary selective enrichment medium (half Fraser broth) to obtain a 10^{-1} dilution (w/v or v/v).
- Incubate at 30°C for 25 h ± 1 hour.

Secondary enrichment:

- Transfer 0.1 ml of the initial suspension to 10 ml of secondary enrichment medium (Fraser broth).
- Incubate at 37°C for 24 h ± 2 hours.

Streaking, isolation and identification:

- Streak the initial suspension in listeria Ottaviani and Agosti agar with an inoculation loop.
- Repeat with the secondary enrichment culture.
- Repeat the operation with the second selective medium (Oxford agar or Palcam agar).
  NOTE: Adjust the incubation conditions to the medium used.
- Incubate the plates of listeria Ottaviani and Agosti agar upside-down at 37°C for 24 h ± 2 hours (and for a further 24 h ± 3 hours, if necessary); the second medium plates should be incubated following the manufacturer's instructions.

NOTE: In the Listeria Ottaviani and Agosti Agar, the *Listeria monocytogenes* colonies are green-blue with an opaque halo around them.

***Listeria spp.* confirmation:**

- Choose five presumptive colonies from each plate and streak them in TSYEA or Blood Agar or Nutrient Agar to obtain isolated colonies.
- Incubate at 37°C for 18-24 hours. The characteristics of the colonies are 1-2 mm, convex, colourless, opaque and with closed edges.
- Proceed with the Catalase test and Gram staining.
- An optional Motility test can be performed, as well as a Voges Proskauer test.

***Listeria monocytogenes* confirmation:**

- Proceed with haemolysis tests and the use of carbohydrates and fats.
- An optional CAMP test can be performed, as well as the motility and catalase tests.

»»» See the culture media references in the table on the next page.

Identification/Confirmation reagents				
Acid Alcohol Decoloriser			064CL00081	
Barrit's reagent (o-Naftol)	RE0100G100			
Crystal violet oxalate		VI0027G100		
Hydrogen peroxide (Catalase)	064-CL0234	HI01351000		
Lugol's solution			064CL00101	
O'Meara's reagent	RE0060G100			
Rhamnose Broth				064-TA8315
Safranine O	SA0042G100			
Xylose Broth				064-TA8316

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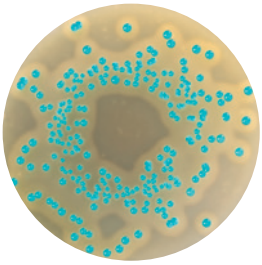
ISO 11290-2 describes a horizontal method for the enumeration of *Listeria monocytogenes*, a pathogen that can be found in food.

Method applicable to:

- Ready-to-eat foods able to support the growth of *Listeria monocytogenes*, other than those intended for infants and for special medical purposes.



Listeria Ottaviani & Agosti Agar
Listeria monocytogenes ATCC 13932

Microbiology of the food chain.
Horizontal method for the detection and enumeration of *Listeria monocytogenes*.
Part 1: Detection method.
Part 2: Enumeration method.

ISO 11290-2 • *Listeria monocytogenes*

PROCEDURE

Preparation of the initial suspension (primary enrichment):

- Add 25 g or ml of the sample to 225 g or ml of buffered peptone water or Fraser broth to obtain a 10^{-1} dilution (w/v or v/v).
- Allow the suspension to stand at 20°C ± 2°C for 1 hour ± 5 minutes.
- If dilutions are used, prepare them from this.

Streaking and incubation:

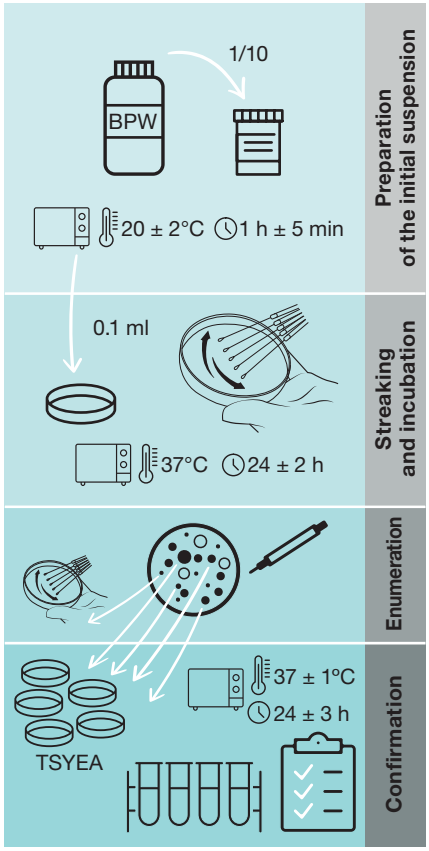
- Streak 0.1 ml of the initial suspension, or the decimal dilutions, in listeria Ottaviani and Agosti agar plates.
- If the number of *Listeria* is presumed to be low, plate 1 ml on three 90 mm plates.
- Incubate at 37°C ± 1°C for 24 h ± 2 hours (plus a further 24 h ± 2 hours).

Enumeration:

- Consider as presumptive *Listeria spp.* any colonies that are blue or greenish with or without an opaque halo. Consider as *Listeria monocytogenes* any colonies that are green-blue with an opaque halo around them. Count both types.
- Go to page 10 for confirmation of *Listeria spp.*

Listeria monocytogenes confirmation:

- Choose five presumptive colonies from each plate and streak them in TSYEA or Blood Agar or Nutrient Agar to obtain isolated colonies.
- Incubate at 37°C ± 1°C for 18-24 hours.
- Proceed with haemolysis tests and the use of carbohydrates and fats.
- An optional CAMP test can be performed, as well as a Motility Test, Catalase Test and Gram Staining.



«««« See the references for the identification/confirmation reagents in the table on the previous page.

Culture media references			90				10V
Blood Agar Base	01-352-500	01-352BA05					
Blood Agar Base No. 2	01-505-500	01-505BA05					
Blood Agar No. 2			064-PA9997				
Buffered Peptone Water (ISO)	02-277-500	02-277BA05		064-BA4010	064-TA0100	064-BA01-2	
				064-BA0486		064-BA01-3	
				064-BA6000		064-BA01-5	
				064-BA6011			
				064-BA6012			
				064-BA8043			
Columbia Blood Agar			064-PA0004				
Columbia Blood Agar Base	01-034-500	01-034BA05					
Fraser Listeria Enrichment Broth Half Fraser with supplement	02-496-500	02-496BA05		064-BA5001	064-TA1125	064-BA02-2	06-111LYO1 [2]
				064-BA5002	064-TA5002	064-BA02-3	06-112LYO1 [3]
						064-BA02-5	06-136LYO1 [4]
							06-145LYO1 [5]
							06-790LYO1 [6]
Listeria Enrichment Broth Base (Lovett)	02-498-500	02-498BA05					06-107LYO1
Listeria Ottaviani & Agosti Agar Microinstant®	01-719-500	01-719BA05	064-PA0084		06-754-024 [1]		06-755LYO1
Methyl Red Voges Proskauer Broth (MRVP Broth)					064-TA0261		
Nutrient agar	01-140-500	01-140BA05	064-PA0059	064-BA1035	064-TA2144		
Oxford Agar Base	01-471-500	01-471BA05					06-127LYO1
TSYEA	02-498-500	02-498BA05					
	07-004-500						

[1] It is an additive
[2] Supplement without ferric citrate for 500 ml of Fraser base broth
[3] Ferric citrate supplement for 500 ml of Fraser Broth / Half Fraser
[4] Supplement for 225 ml of Half Fraser base broth
[5] Supplement for 500 ml of Half Fraser base broth
[6] Ferric supplement for 500 ml of Fraser base broth

Escherichia coli • ISO 16649-1/2

Microbiology of the food chain.
Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli*.

Part 1: **Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl ß-D-glucuronide.**

Part 2: **Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl ß-D-glucuronide.**

Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide.

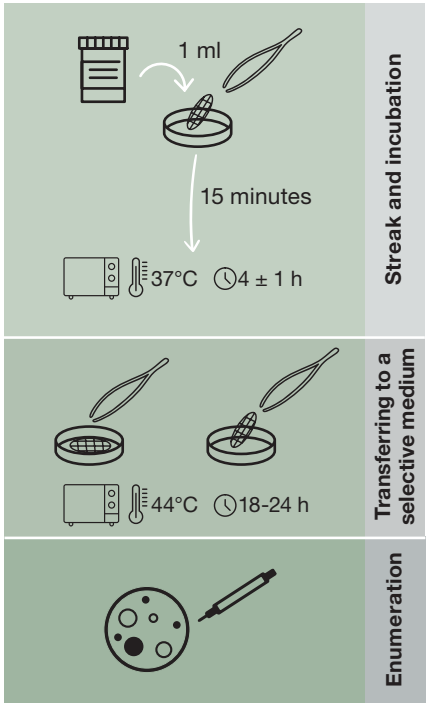
This international regulation specifies a horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* through the counting technique with and without membrane and the detection using the most probable number technique. This applies to:

Part 1 and/or 2:

- Minced meat.
- Mechanically separated meat (MSM).
- Meat preparations.
- Shelled and shucked products of cooked crustaceans and molluscan shellfish.
- Cheeses made from milk or whey that has undergone heat treatment.
- Cheeses made from milk that has undergone pasteurisation or a lower heat treatment and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment.
- Unripened soft cheese made from milk or whey that has undergone pasteurisation or a stronger heat treatment.
- Butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation.
- Pre-cut fruit and vegetables (ready-to-eat).
- Unpasteurised fruit and vegetable juices (ready-to-eat).

PROCEDURE

Part 1:



Streak and incubation:

- With sterile tweezers, place the membranes over the surface of two Minerals Modified Glutamate Agar Base plates.
- Add 1 ml of the initial sample suspension to the centre of each membrane and spread. Repeat the process with the next dilutions.
- Allow the plates to stand at room temperature for 15 minutes, then incubate at 37°C for 4 h ± 1 hours.

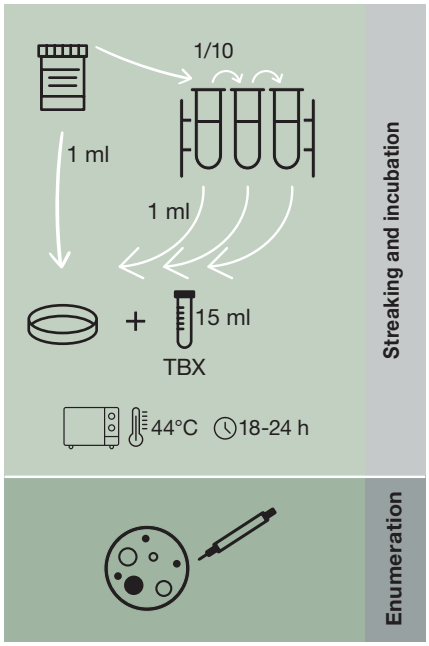
Transferring to a selective medium:

- With sterile tweezers, transfer the membranes from the Minerals Modified Glutamate Agar Base plates to the TBX agar plates.
- Incubate the plates at 44°C for 18-24 hours.

Enumeration:

- Count the typical CFU from ß-glucuronidase-positive Escherichia coli in plates with over 150 typical CFU.

Part 2:



Streaking and incubation:

- Transfer 1 ml of the sample (if liquid) or 1 ml of the initial dilution (10^{-1}), for other product, to empty, sterile Petri dishes.
- Inoculate two plates for each dilution.
- Add 15 ml of TBX medium previously cooled in a water bath at 42-47°C to each plate.
- Incubate at 44°C for 18-24 hours.

Enumeration:

- Count the typical CFU from ß-glucuronidase-positive *Escherichia coli* in plates with over 150 typical CFU.

»»»» See references in the table on the following page.

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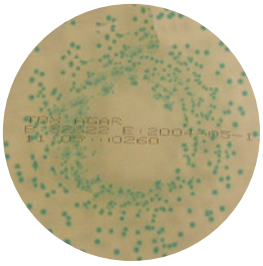
ISO 16649-3 • *Escherichia coli*

This international regulation specifies a horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* through the counting technique with and without membrane and the detection using the most probable number technique. This applies to:

Part 3:
- Live bivalve molluscs, echinoderms, tunicates and marine gastropods.
- Shelled and shucked products of cooked crustaceans and molluscan shellfish.



TBX Agar
Escherichia coli ATCC 25922

Microbiology of the food chain.
Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli.*

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

Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl ß-D-glucuronide.

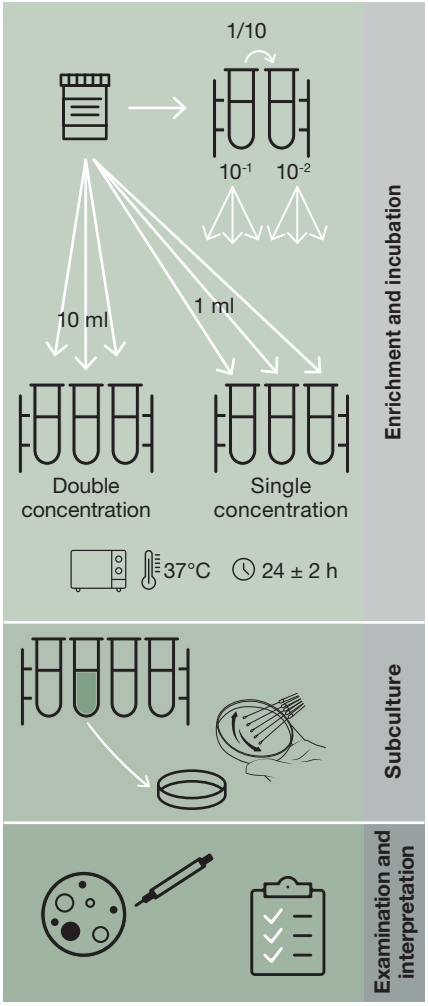
Part 3: **Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide.**

PROCEDURE

Part 3:

Enrichment and incubation:

- Add 10 ml of the sample (if liquid) or 10 ml of the initial suspension (for other products) to 3 tubes with 10 ml of **double concentration** Minerals Modified Glutamate Medium Broth.
- Add 1 ml of the sample (if liquid) or 1 ml of the initial suspension (for other products) to 3 tubes with 10 ml of **single concentration** Minerals Modified Glutamate Medium Broth.
- For the other dilutions (10^{-1}, 10^{-2}) add 1 ml of the sample to 3 tubes with 10 ml of single concentration Minerals Modified Glutamate Medium Broth.
- Incubate at 37°C ± 1°C for 24 h ± 2 hours.



Subculture:

- From the tubes with yellow color (acid presence), streak to TBX plates to isolate colonies with an 10 µl inoculation loop.
- Incubate at 44°C ± 1°C for 21 h ± 3 hours.

Examination and interpretation:

- Look for colonies with the typical blue/blue-green colour.
- Consider tubes with single or double concentration to be positive, which produce blue colonies in the selective medium after incubation and subculture.

						Supplements
Minerals Modified Glutamate Agar Base	01-659-500	01-659BA05				
						AM02730500
						SO04000500
Minerals Modified Glutamate Medium Base	02-656-500	02-656BA05			064-TA8312	
					064-TA8311 double concentration	AM02730500
						SO04000500
Tryptone Bile Glucuronic Agar (TBX Agar) Microinstant®	01-619-500	01-619BA05	064-PA0945	064-BA0945		

Enterobacteriaceae • ISO 21528-1

Microbiology of the food chain.

Horizontal method for the detection and enumeration of *Enterobacteriaceae.*

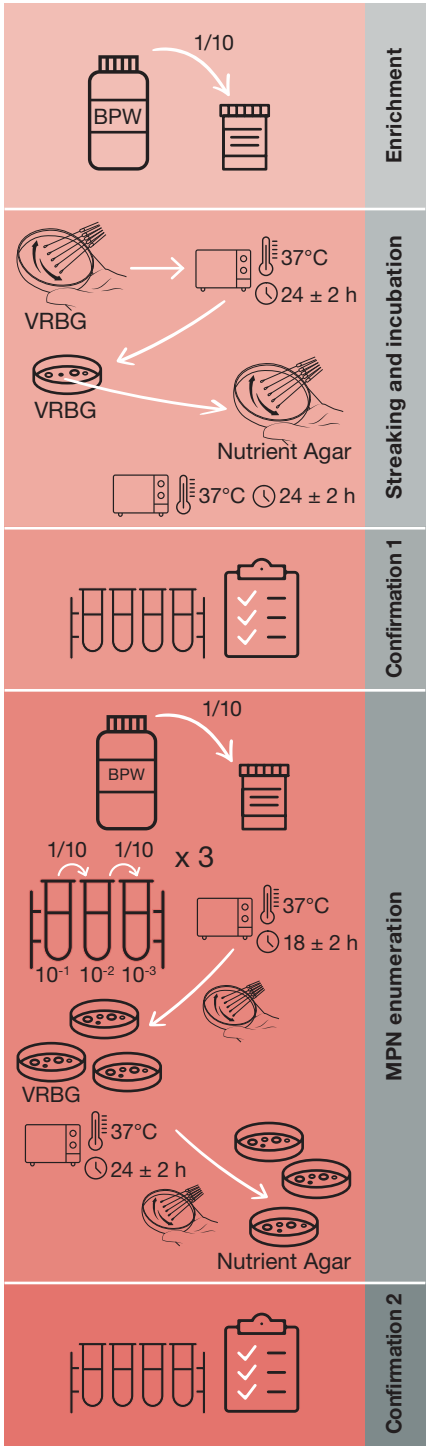
Part 1: Detection of *Enterobacteriaceae.*

Part 2: Colony-count technique.

This part of ISO 21528 (Part 1) describes a horizontal method for the detection and enumeration of *Enterobacteriaceae* through the most probable number technique.

Method applicable to:

- Pasteurised milk and other pasteurised liquid dairy products.
- Dried infant formula and dried foods for special medical purposes intended for infants below 6 months of age.
- Dried follow-on formula.



PROCEDURE

Part 1:

Enrichment:

- 10^{-1} dilution (v/v or w/v) of the sample in buffered peptone water (BPW).
- Incubate at 37°C for 18 h ± 2 hours.

Streaking and incubation:

- Streak in VRBG agar plates from the enrichment medium with a 10 µl inoculation loop.
- Incubate at 37°C for 24 h ± 2 hours.
- Select at least one colony with the correct characteristics: pinky purple, with or without a halo of precipitation; or four presumptive colonies in Nutrient Agar.
- Incubate at 37°C for 24 h ± 2 hours.

Confirmation 1 and 2:

- Confirm using the oxidase (negative) and glucose fermentation (positive) tests with incubation in tubes at 37°C for 24 h ± 2 hours.

MPN enumeration (Annex):

- Add x g of the sample to 9 x ml of BPW and homogenise.
- Prepare a dilution bank in tubes from 10^{-1} to 10^{-3}, prepare in triplicate.
- Incubate the nine tubes at 37°C for 18 h ± 2 hours.
- Streak the nine dilutions on VRBG agar plates.
- Incubate at 37°C for 24 h ± 2 hours.
- Streak the characteristic colonies in nutrient agar.
- Confirm using the oxidase (negative) and glucose fermentation (positive) tests with incubation in tubes at 37°C for 24 h ± 2 hours.

Buffered Peptone Water (ISO)	02-277-500	
Nutrient Agar (ISO)	01-635-500	
Oxidase test	RE00650005	
Oxidation-Fermentation Fluid Medium Base (O/F Medium)		
Violet Red Bile Dextrose Agar (VRBD AGAR) (Eur. Pharm.)	01-295-500	

[1] Sterile vaseline

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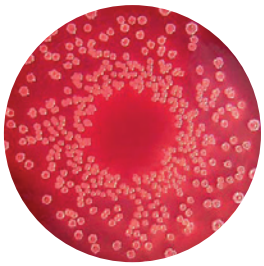
ISO 21528-2 • Enterobacteriaceae

This part of ISO 21528 (Part 2) describes a horizontal method for the enumeration of *Enterobacteriaceae* through the colony-count technique.

Method applicable to:

- Carcasses of cattle, sheep, goats and horses.
- Carcasses of pigs.
- Powdered milk and whey powder.
- Ice-cream and frozen dairy desserts.
- Egg products.



VRBG
Escherichia coli ATCC 25922

Microbiology of the food chain.

Horizontal method for the detection and enumeration of *Enterobacteriaceae*.

Part 1: Detection of *Enterobacteriaceae*.

Part 2: **Colony-count technique.**

PROCEDURE

Part 2:

Initial suspension:

- From a liquid sample, prepare decimal dilutions in buffered peptone water (BPW). For other types of sample, prepare an initial suspension which will be used to prepare the decimal dilutions with BPW.

Streaking:

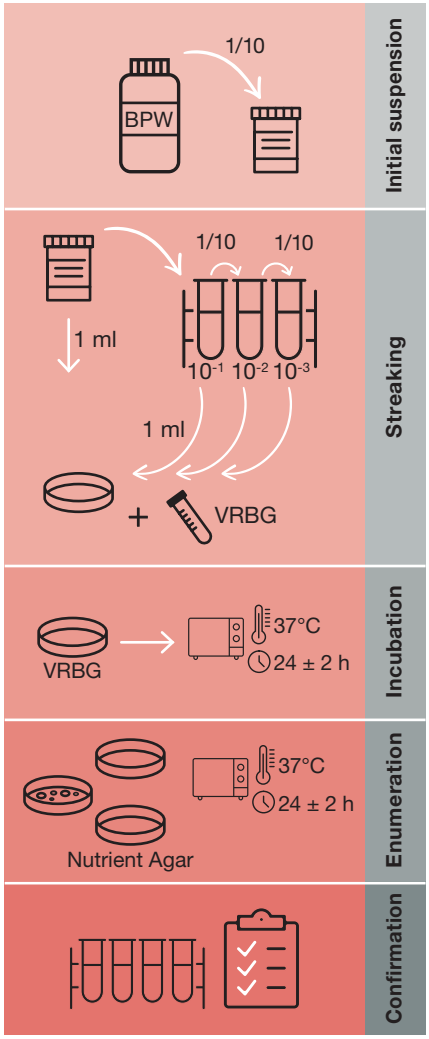
- For the liquid samples, transfer 1 ml of the sample to an empty Petri dish and 1 ml of the 10^{-1} dilution to another empty petri dish; apply the same procedure to all the other dilutions. Follow the same procedure for the other types of sample.
- Pour 15 ml of VRBG (between 47°C and 50°C) onto each plate, mix and allow to solidify.
- Once solidified, add an extra layer of 5-10 ml of VRBG and allow to solidify.

Incubation:

- Incubate at 37°C for 24 h ± 2 hours.

Enumeration and confirmation:

- Select plates with less than 150 colonies with the following characteristics: pinky purple with or without a halo.
- Streak again, at least five colonies, in Nutrient Agar.
- Incubate at 37°C for 24 h ± 2 hours.
- Confirm using the oxidase (negative) and glucose fermentation (positive) tests.



	90				Swabs
02-277BA05		064-BA4010	064-TA0100	064-BA01-2	
		064-BA0486		064-BA01-3	
		064-BA6000		064-BA01-5	
		064-BA6011			
		064-BA6012			
		064-BA8043			
01-635BA05					
					06-120-050
		06-077-100[1]	064-TA8327		
01-295BA05	064-PA0046	064-BA1011	064-TA0147		
		064-BA3061			

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ISO 22964 • *Cronobacter spp.*

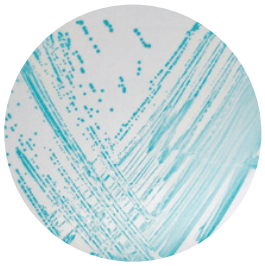
Microbiology of the food chain.

Horizontal method for the detection of *Cronobacter spp.*

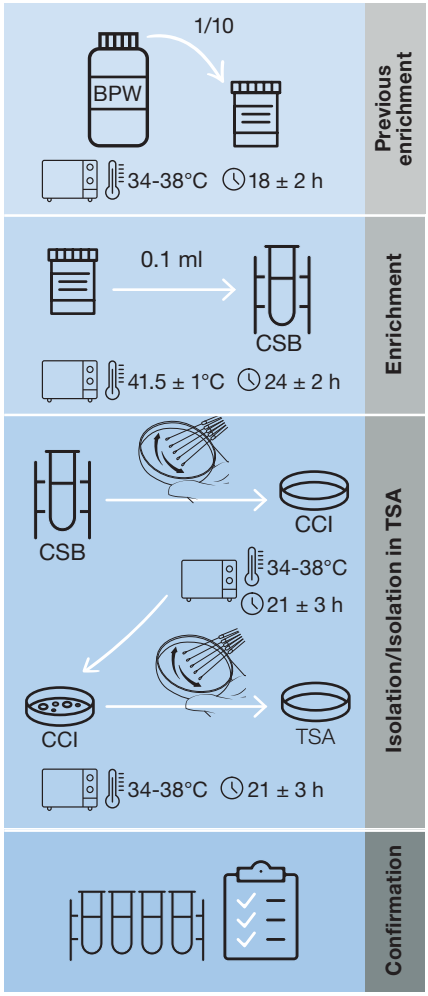
ISO 22964 describes a horizontal method for the detection of *Cronobacter spp.* in food and in environmental samples.

Method applicable to:

- Dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age.



CCI
Cronobacter sakazakii ATCC 29544



PROCEDURE

Previous enrichment:

- 10^{-1} dilution (v/v or w/v) of the sample in Buffered Peptone Water (BPW).
- Incubate at 34-38°C for 18 h ± 2 hours.

Enrichment:

- Add 0.1 ml of the previous enrichment to 10 ml of Cronobacter selective broth base (CBS).
- Incubate at 41.5°C for 24 h ± 2 hours.

Isolation:

- Streak as for isolation in Chromogenic Cronobacter Isolation Agar (CCI).
- Incubate at 41.5°C ± 1°C for 24 h ± 2 hours.

Isolation in TSA:

- Select 5 typical colonies of *Cronobacter spp.* in CCI (small to medium, 1-3 mm, blue or greenish blue).
- White colonies with or without a green, grey or black centre are not *Cronobacter.* Neither are the yellow or red ones.
- Streak the five suspicious or typical colonies in TSA.
- Incubate at 34-38°C for 21 h ± 3 hours.

Confirmation:

- Biochemical tests for the confirmation are:
  - Hydrolysis of the substrate 4-nitrophenyl α-D-glucopiranoside
  - L-Lysine decarboxylation
  - L-Ornithine decarboxylation
  - Carbohydrates fermentation: D-arabitol, D-sorbitol, D-sucrose and α-methyl-D-glucoside (optional)
  - Oxidase
  - Methyl red (optional)
  - Voges-Proskauer (optional)

									Swabs
Buffered Peptone Water (ISO)		02-277-500	02-277BA05		064-BA4010	064-TA0100	064-BA01-2		
					064-BA0486		064-BA01-3		
					064-BA6000		064-BA01-5		
					064-BA6011				
					064-BA6012				
					064-BA8043				
Cronobacter Selective Broth (CSB)		02-827-500						06-712LYO1	
Microinstant® Chromogenic Cronobacter Isolation Agar (CCI)		01-828-500							
Oxidase Test	RE00650005								06-120-050
Tryptic Soy Agar (TSA)		01-200-500	01-200BA05	064-PA0031	064-BA1008	064-TA0121			
				064PA0031I	064-BA6043	064-TA0221			
				064-PA0138	BA1008-500				

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ISO 11133

This standard specifies the parameters to be analysed and the procedures to conduct to determine the correct performance of the culture media as described in the international ISOs applicable to microbiology of food, animal feed and water.

It applies to:
- Commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media;
- Non-commercial bodies supplying media to third parties;
- Microbiological laboratories preparing culture media for their own use.

Microbiology of food, animal feed and water.
Preparation, production, storage and performance testing of culture media.

The main aim of laboratories carrying out microbiological tests is to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms.

The tests and procedures depend on culture media being able to provide reproducible and consistent results. So, a main requirement is that culture media must meet the established performance criteria. Therefore, a sufficient number of experiments should be carried out to demonstrate:

a) the acceptability of each batch of medium,
b) that the medium "fit for purpose",
c) that the medium can produce consistent results.

These three criteria make an essential part of internal quality control procedures and, with appropriate documentation, will permit effective monitoring of culture media and contribute to the production of both accurate and reliable data. For all media described in standard methods, it is essential to define the minimum acceptance criteria required to ensure their reliability and therefore it is recommended that tests to determine the performance characteristics of a culture media are conducted following the international standard **ISO 11133:2014/A1:2018.**

The requirements of international standard **ISO 11133:2014/A1:2018** have precedence in the assessment of culture media quality for the microbiological analysis of food, animal feed and water. The acceptance criteria determined in this standard can be used in all microbiological laboratories to evaluate the productivity, selectivity and/or electivity of a culture medium.

The object and scope of standard **ISO 11133:2014/A1:2018** describes the terms related to quality assurance of culture media and specifies the requirements for their preparation and usage for the microbiological analysis of the food, animal feed and water.

These requirements are applicable to all categories of culture media prepared for use in laboratories performing microbiological analyses.

International standard **ISO 11133:2014/A1:2018** establishes criteria and describes methods for the performance testing of culture media and it applies to producers such as:

- Commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media;
- Non-commercial bodies supplying media to third parties;
- Microbiological laboratories preparing culture media for their own use.

To this end, the table on the following page shows the tests to perform and the test microorganisms to use in the quality control of the culture media described in the ISO standards for the control of the microbiological parameters included in the **COMMISSION REGULATION (EC) No. 1441/2007 of 5 December 2007 amending REGULATION (EC) No. 2073/2005** on microbiological criteria for foodstuffs, and subsequent additions.

»»»» See tests and test microorganisms table on the next page.

	Function	Incubation	Microorganism	Reference	Medium of reference	Control method	Criteria	Characteristic reaction
ISO 4833								
PCA MPCA	Productivity	(72 ± 3) h/ (30 ± 1) °C	*Bacillus subtilis subsp. spizizenii* ATCC® 6633™/ WDCM 00003	P10006-MSC	TSA	Quantitative	P R ≥ 0.7	–
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	TSA			–
			Staphylococcus aureus ATCC® 25923™ / WDCM 00034	660-00360P	TSA			–
ISO 6579								
Nutrient Agar	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	Good growth (2)	–
			Salmonella enteriditis ATCC® 13076™ / WDCM 00030	660-00345P	–			–
BPW	Productivity	(18 ± 2) h/ (37 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	Turbidity (1-2)	–
			Salmonella enteriditis ATCC® 13076™ / WDCM 00030	660-00345P	–			–
MKTT	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	> 10 colonies in XLD or other election medium	Characteristic colonies for each medium (see ISO 6579 standard)
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Pseudomonas aeruginosa ATCC® 27853™ / WDCM 00025	660-00353C	–			
	Selectivity	(24 ± 3) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Parcial inhibition ≤ 100 colonies in TSA	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–		< 10 colonies in TSA	–
MSRV	Productivity	2 × (24 ± 3) h/ (41.5 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	White or greyish turbid zone, which spreads to the outside from the inoculated drops. After a time of 24-48 h, the turbid zones will occupy (mostly) all the extension of the plate.	Possible extra: characteristic colonies after the subculture in XLD k
	Selectivity	2 × (24 ± 3) h/ (41.5 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Possible growth in the place of the drop inoculation without turbid zones.	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–		No growth	–
RVS	Productivity	(24 ± 3) h/ (41.5 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	> 10 colonies in XLD or other election medium	Characteristic colonies for each medium (see ISO 6579 standard)
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Pseudomonas aeruginosa ATCC® 27853™ / WDCM 00025	660-00353C	–			
	Selectivity	(24 ± 3) h/ (41.5 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Partial inhibition ≤ 100 colonies in TSA	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–		< 10 colonies in TSA	–
XLD	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Salmonella enterica serovar Typhimurium* ATCC® 14028™/ WDCM 00031	P10010-MSC	–	Qualitative	Good growth (2)	Colonies with a black center and slightly transparent red zone due to the medium's change of color
	Selectivity	(24 ± 3) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	S00004-MSC	–	Qualitative	Growth or partial inhibition (0-1)	Yellow colonies
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–		Total inhibition (0)	–
ISO 6888-1								
Baird-Parker	Productivity	de (24 ± 2) h a (48 ± 2) h / (37 ± 1) °C	*Staphylococcus aureus* ATCC® 25923 /WDCM 00034	660-00360A	TSA	Quantitative	P R ≥ 0.5	Grey or black colonies with clear halo (Egg yolk clarification reaction)
			Staphylococcus aureus subsp. aureus ATCC® 6538™/ WDCM 00032	P10003-MSC				
	Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition (0)	–
	Specificity	de (24 ± 2) h a (48 ± 2) h / (37 ± 1) °C	*Staphylococcus saprophyticus* ATCC® 15305 / WDCM 00159	660-00494A	–	Qualitative	–	Grey or black colonies without Egg yolk clarification reaction
			Staphylococcus epidermidis ATCC® 12228™/ WDCM 00036	S00014-MSC				
ISO 6888-1/ISO 6888-3								
BHI	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Staphylococcus aureus* ATCC® 25923™ / WDCM 00034	660-00360A	–	Qualitative	Turbidity (1-2) f	–
ISO 6888-2								
RPFA	Productivity	de (24 ± 2) h a (48 ± 2) h / (37 ± 1) °C	*Staphylococcus aureus* ATCC® 25923 /WDCM 00034	660-00360A	TSA	Quantitative	P R ≥ 0.5	Grey or black colonies with opaque halo
			Staphylococcus aureus subsp. aureus ATCC® 6538™/ WDCM 00032	P10003-MSC				
	Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition (0)	–
	Specificity	de (24 ± 2) h a (48 ± 2) h / (37 ± 1) °C	*Staphylococcus saprophyticus* ATCC® 15305 / WDCM 00159	660-00494P	–	Qualitative	–	Grey or black colonies without opaque halo
			Staphylococcus epidermidis ATCC® 12228™/ WDCM 00036.	S00014-MSC				
ISO 7932								
MYP	Productivity	(24 ± 3) h a (44 ± 4) h/ (30 ± 1) °C	*Bacillus cereus* ATCC® 11778™/ WDCM 00001	S00015-MSC	TSA	Quantitative	P R ≥ 0.5	Pink colonies with precipitation halo
	Selectivity	(44 ± 4) h/ (30 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition (0)	–
	Specificity	(44 ± 4) h/ (30 ± 1) °C	*Bacillus subtilis subsp. spizizenii* ATCC® 6633™/ WDCM 00003	S00006-MSC	–	Qualitative	–	Yellow colonies without precipitation halo
ISO 10272 (all parts)								
mCCDA	Productivity	(44 ± 4) h/ (41.5 ± 1) °C microaerophilic atosmphere	*Campylobacter jejuni subsp. jejuni* ATCC® 33291™/ WDCM 00005	S00018-MSC	–	Qualitative	Good growth (2)	Greyish, flat and moisty, sometimes with metallic shimmer
			Campylobacter jejuni subsp. jejuni ATCC® 29428™/ WDCM 00156	660-00325P				
	Selectivity	(44 ± 4) h/ (41.5 ± 1) °C microaerophilic atosmphere	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total or partial inhibition (0-1)	There are no characteristic colonies
			Staphylococcus aureus ATCC® 25923 / WDCM 00034	660-00360P	–	Qualitative	Total inhibition (0)	–
ISO 10272-2								
Blood Agar	Productivity	(44 ± 4) h/ (41.5 ± 1) °C	*Campylobacter jejuni subsp. jejuni* ATCC® 33291™/ WDCM 00005	S00018-MSC	Lot of blood agar medium already validated	Quantitative	P>= 0.7	–
			Campylobacter jejuni subsp. jejuni ATCC® 29428™/ WDCM 00156	660-00325P				
mCCDA	Productivity	(44 ± 4) h/ (41.5 ± 1) °C microaerophilic atosmphere	*Campylobacter jejuni subsp. jejuni* ATCC® 33291™/ WDCM 00005	S00018-MSC	TSA	Quantitative	P R ≥ 0.5	Greyish, flat and moisty, sometimes with metallic shimmer
			Campylobacter jejuni subsp. jejuni ATCC® 29428™/ WDCM 00156	660-00325P				
	Selectivity	(44 ± 4) h/ (41.5 ± 1) °C microaerophilic atosmphere	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	–	–
			Staphylococcus aureus ATCC® 25923 / WDCM 00034	660-00360P	–		–	–

ISO 11290 (all parts)								
TSYEA	Productivity	(21 ± 3) h/ (37 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	–	Qualitative	Good growth (2)	–
TSYEB	Productivity	(21 ± 3) h/ (25 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	–	Qualitative	Turbidity (1-2) f	–
ISO 11290-1 (all parts)								
Listeria Agar according to Ottaviani & Agosti	Productivity	(44 ± 4) h/ (37 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	–	Qualitative	Good growth (2)	Blueish green colonies with opaque halo
	Selectivity	(44 ± 4) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition (0)	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC				
	Specificity	(44 ± 4) h/ (37 ± 1) °C	*Listeria innocua* ATCC® 33090™/ WDCM 00017	S00019-MSC	–	Qualitative	–	Blueish green colonies without opaque halo
Fraser	Productivity	(48 ± 2) h/ (37 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	–	Qualitative	> 10 colonies in Listeria Agar according to Ottaviani & Agosti	Blueish green colonies with opaque halo
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–			
			Listeria monocytogenes ATCC® 35152™/ WDCM 00109	660-01190P	–			
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–			
	Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition in TSA	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087.	S00008-MSC	–	Qualitative	< 100 colonies in TSA	–
Half-Fraser	Productivity	(24 ± 2) h/ (30 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	–	Qualitative	> 10 colonies in Listeria Agar according to Ottaviani & Agosti	Blueish green colonies with opaque halo
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–			
			Listeria monocytogenes ATCC® 35152™/ WDCM 00109	660-01190P	–			
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC	–			
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–			
	Selectivity	(24 ± 2) h/ (30 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition in TSA	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	< 100 colonies in TSA	–
ISO 11290-2 (all parts)								
Listeria Agar according to Ottaviani & Agosti	Productivity	(44 ± 4) h/ (37 ± 1) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	TSA	Quantitative	P R ≥ 0.5	Blueish green colonies with opaque halo
	Selectivity	(44 ± 4) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 or *Escherichia coli* ATCC® 25922™/ WDCM 00013. Contains two swabs	S00004-MSC or S00009-MSC	–	Qualitative	Total inhibition (0)	–
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC	–			
	Specificity	(44 ± 4) h/ (37 ± 1) °C	*Listeria innocua* ATCC® 33090™/ WDCM 00017	S00019-MSC	–	Qualitative	–	Blueish green colonies without opaque halo
BPW	Dilución	(1 h ± 5 min)/ (20 ± 2) °C	*Listeria monocytogenes* ATCC® 13932™/ WDCM 00021	S00020-MSC	TSA	Quantitative	± 30% of colonies/T0 (± 30% of the original count)	–
ISO 16649-1 and ISO 16649-2								
TBX	Productivity	(21 ± 3) h/ (44 ± 1) °C	*Escherichia coli* ATCC® 25922™/ WDCM 00013	S00009-MSC	TSA	Quantitative	P R ≥ 0.5	Blue colonies
	Selectivity	(21 ± 3) h/ (44 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	Total inhibition (0)	–
	Specificity	(21 ± 3) h/ (44 ± 1) °C	*Citrobacter freundii* ATCC® 43864™/ WDCM 00006	S00023-MSC	–	Qualitative	–	White to greenish beige colonies
ISO 16649-3								
MMG	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	P10004-MSC	–	Qualitative	Acid production	Change to yellow colour
	Selectivity	(24 ± 2) h/ (37 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	No growth	–
TBX	Productivity	(21 ± 3) h/ (44 ± 1) °C	*Escherichia coli* NCTC 13216 / WDCM 00202	660-01192P	–	Qualitative	Good growth (2)	Blue colonies
			Escherichia coli ATCC® 8739™/ WDCM 00012	P10004-MSC				
	Selectivity	(21 ± 3) h/ (44 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	Total inhibition (0)	–
	Specificity	(21 ± 3) h/ (44 ± 1) °C	*Citrobacter freundii* ATCC® 43864™/ WDCM 00006	S00023-MSC	–	Qualitative	–	White to greenish beige colonies
ISO 21528 (all parts)								
Nutrient Agar	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	P10004-MSC	–	Qualitative	Good growth (2)	–
ISO 21528-1 (all parts)								
BPW	Productivity	(18 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	P10004-MSC	–	Qualitative	Turbidity (1-2)	–
			Salmonella enterica serovar Typhimurium ATCC® 14028™/ WDCM 00031	P10010-MSC				
EE	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012 + *Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	P10004-MSC	–	Qualitative	> 10 colonies in VRBG	Pink to red colonies with or without precipitation halo
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC				
			Salmonella enterica serovar Typhimurium ATCC® 14028™/ WDCM 00031	P10010-MSC				
			Enterococcus faecalis ATCC® 29212™/ WDCM 00087	S00008-MSC				
	Selectivity	(24 ± 2) h/ (37 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Total inhibition (0) in TSA	–	–
VRBG	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	P10004-MSC	–	Qualitative	Good growth (2)	Pink to red colonies with or without precipitation halo
			Salmonella enterica serovar Typhimurium ATCC® 14028™/ WDCM 00031	P10010-MSC				
	Selectivity	(24 ± 2) h/ (37 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	Total inhibition (0)	–
ISO 21528-2 (all parts)								
VRBG	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* ATCC® 8739™/ WDCM 00012	P10004-MSC	TSA	Quantitative	P R ≥ 0.5	Pink to red colonies with or without precipitation halo
			Salmonella enterica serovar Typhimurium ATCC® 14028™/ WDCM 00031	P10010-MSC				
	Selectivity	(24 ± 2) h/ (37 ± 1) °C	*Enterococcus faecalis* ATCC® 29212™/ WDCM 00087	S00008-MSC	–	Qualitative	Total inhibition (0)	–

scharlab.com

Scharlab S.L.
Gato Pérez, 33. Pol. Ind. Mas d'en Cisa.
08181 Sentmenat, Barcelona, Spain
Tel.: +34 93 715 19 40 - Fax: +34 93 715 27 65
E-mail: helpdesk@scharlab.com

Scharlab Italia S.r.l.
Via Alcide De Gasperi 56.
20070 Riozzo Di Cerro al Lambro (Mi), Italy
Tel.: +39 02 9823 0679 / +39 02 9823 6266
Fax: +39 02 9823 0211 / +39 02 9811 9288
E-mail: customerservice@scharlab.it

Scharlab Philippines, Inc.
4/F Unit K, No. 35 Sto. Niño Street corner Roosevelt Ave.
Barangay San Antonio, Quezon City 1105, Philippines.
Tel. - Fax: + 63 2 529 5726
E-mail: infophilippines@scharlab.ph

Scharlab Brasil S/A
Estrada do Campo Limpo, 780.
São Paulo. 05777-000, Brasil
Tel.: (11) 5512 5744 - Fax: (11) 5511 9366
E-mail: mkt@scharlab.com.br

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