



PROTOCOLS

**MICROBIOLOGICAL  
ANALYSIS  
IN THE FOOD  
INDUSTRY**

## How important are the reference methods in food control?

Many of the foods that do not undergo a microbiological analysis become a health risk, because they can cause a wide range of diseases. Diarrhoeal diseases, for example, are the leading cause of death in children and the second in adults, and in many cases they are related to the intake of a contaminated food. That is why it is necessary to establish microbiological criteria to ensure the safety of food and protect the health of the consumer.

The development of **criteria for the microbiological control of foods** implies, among other things, fixing the analytical method to be used to ensure compliance with these criteria. These methods, developed by organizations such as ISO or CEN, can be considered as reference methods, and their implementation and application in the laboratory can often be difficult.

Currently, the strategy in the development and update of these **ISO methods** aims at improving them through the simplification and incorporation of new technologies, so that they can be routinely used in food control laboratories.

The use of standardized methods for conducting microbiological analyses is a tool that allows laboratories to use **internationally recognized testing methods**, thus contributing to the production of comparable results, without sacrificing reliability and ensuring the quality and safety of the foods subject to control.

That is why it is increasingly common for these standardized methods to be taken as a reference by accreditation bodies as well as to establish microbiological criteria (EU Regulations 2073/2005 and 1441/2007) where **the international standards developed by the ISO and CEN Committees are considered reference methods**.

At **Condalab** we want to help the different quality control laboratories by making available the full range of culture media under **ISO** formulations as well as their **different analysis procedures**.



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# Mesophilic Microorganisms count

Procedure as defined by ISO 4833:2013

## Introduction

Bacteria are considered the main cause of diseases that have the consumption of contaminated food as their root cause. When foods show variations or a change in colour, texture or consistency, it is a sign of deterioration and possible contamination. To grow, bacteria need:

- Nutrients such as carbon, nitrogen, hydrogen and phosphorus (among others) that are available in food.
- Water as a fundamental element, because in its absence, bacterial growth stops.
- A suitable pH, oscillating around a neutral value (6-7). At a lower or higher pH, bacterial growth stops.

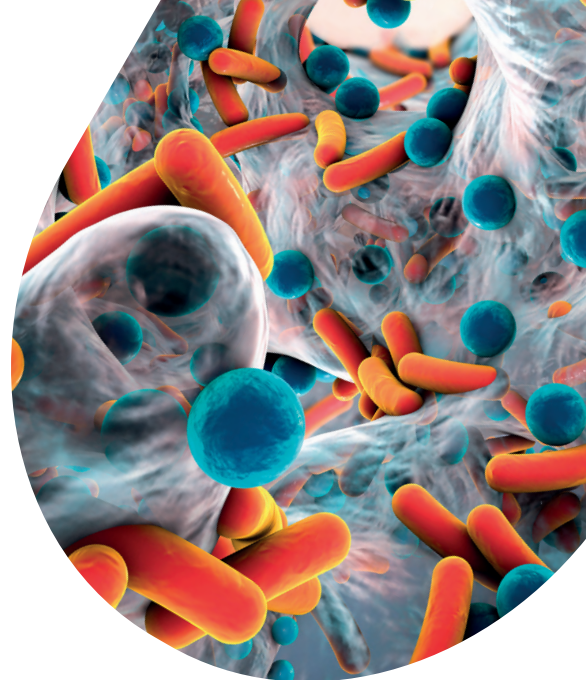
The analysis of this group of bacteria includes all microorganisms capable of developing in the presence of oxygen at a temperature between 20 °C and 45 °C. The count of aerobic mesophilic microorganisms, under the established conditions, estimates the total microflora of the product but without specifying and identifying the type of microorganism.

## Bibliography

AFSA, 2001. *Evaluation of microbiological methods for detection and for enumeration of microbiological contaminants in food. Final report Contract SMT4/CT96 2098. Coordination by Agence Française de Sécurité Sanitaire des Aliments. AFSSA, France, February 2001.*

ISO 4833-1:2013. *Microbiology of the food chain – Horizontal method for the enumeration of microorganisms. Part 1: Colony-count at 30 degrees C by the pour plate technique.*

ISO 4833-2:2013. *Microbiology of the food chain – Horizontal method for the enumeration of microorganisms. Part 2: Colony-count at 30 degrees C by the surface plate technique.*



## Method

### INITIAL SUSPENSION

Refer to the **ISO 6887-1** specific to the product that is analyzed

### COUNTING MEDIUM

1 ml sample diluted in Standard Methods Agar (PCA) (**CAT. 1056**) Incubation: 30 °C ± 1 °C // 72 ± 3 h

**For the analysis of dairy products, the culture medium should carry 1 g/l of skimmed milk powder: Standard Methods Agar with Powdered Milk (CAT. 1033)**

**NOTE:** \*For this standard, two methods can be distinguished: Pour plate (4833-1) and spread plate (4833-2) techniques. Both sections of the standard establish the same working protocol but warn that, for certain matrices, different results may be obtained according to the section followed.

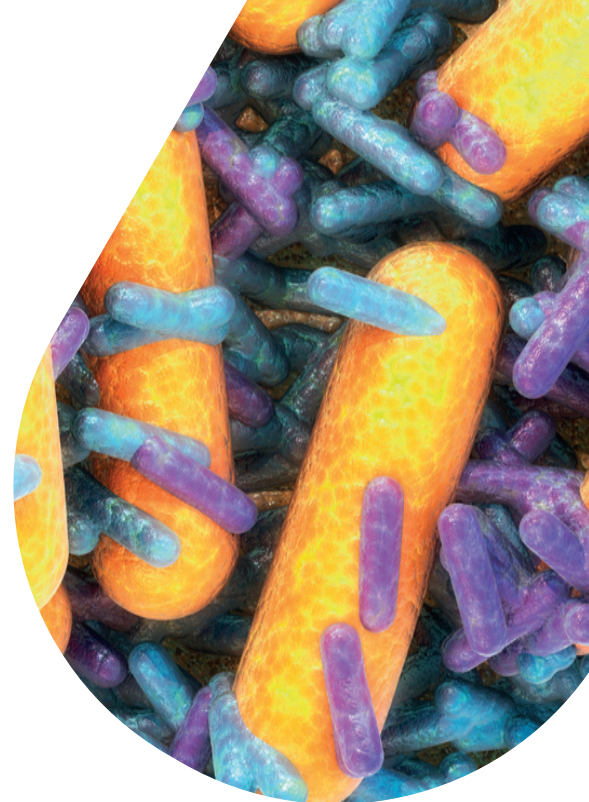
# Mold and Yeast

Procedure as defined by ISO 21527 1:2008

## Introduction

Foods that have fungi on their surface may have toxins in their interior, constituting a risk of allergic reactions. When fungi appear on the surface of a food, their roots have already invaded a significant portion of the product. Fungi live on plant or animal matter and contain spores that can be transported by air, water or insects.

Fungi are found mostly in foods such as fruit, vegetables, moist bread or cheeses. Different types can be distinguished depending on their behaviour. *Aspergillus* or *Penicillium* are some of the fungi most frequently found. The filaments in their structure, the hyphae, produce enzymes that can break down the hardest molecules. Under suitable conditions (warm temperatures and humidity), fungi produce mycotoxins, substances with the capacity to cause diseases and that appear mainly in cereals and nuts.



## Bibliography

VALERIE T., MICHAEL T.S., PHILIP B., MISLIVEC, HERBERT A.K. AND RUTH B. BAM: *Yeast, Molds and Mycotoxins*. US FOOD AND DRUG ADMINISTRATION.

ISO 21527-1:2008. *Microbiology of the food chain – Horizontal method for the enumeration of yeast and molds. Part 1: Colony count technique in products with water activity greater than 0,95.*

ISO 21527-2:2008. *Microbiology of the food chain – Horizontal method for the enumeration of yeast and molds. Part 1: Colony count technique in products with water activity less than or equal 0,95.*

### PRODUCTS WITH WATER ACTIVITY > 0,95 (ISO 21527-1)

#### INITIAL SUSPENSION

Except for preparations of specific samples (ISO 6887-1), dilution in Buffered Peptone Water (CAT. 1402) is recommended

#### COUNTING MEDIUM

0.1 ml sample diluted in Rose Bengal Agar + Cholamphenicol + Dichloran (DRBC Agar) (CAT. 1160)  
Incubation: 25 °C ± 1 °C // 5 days

### PRODUCTS WITH WATER ACTIVITY ≤ 0,95 (ISO 21527-2)

#### INITIAL SUSPENSION

Refer to the ISO 6887-1 standard specific to the product to be analysed. Except for preparations involving specific samples, dilution in Buffered Peptone Water (CAT. 1402) is recommended

#### COUNTING MEDIUM

0.1 ml sample diluted in Dichloran Glycerol Agar (DG 18) (CAT. 1161) / Incubation: 25 °C ± 1 °C / 5 – 7 days

# Detection of *Salmonella* spp

Procedure as defined by ISO 6579:2017

## Introduction

*Salmonella* is a very diverse family of microorganisms, including about 2,300 different serotypes. It is estimated that this family of bacteria is the cause of millions of hospitalizations in the world (1.2 million in the USA alone), but this number would increase up to 30 times if undiagnosed infections were taken into account, as they usually cause a normal diarrhoeal process.

This group of bacteria is gram-negative (since they belong to the large group of Enterobacteriaceae), facultative anaerobic, and motile by means of flagella. They are glucose fermenters, but they are not lactose fermenters and do not produce urease.

*Salmonella* is present in the intestines of people and animals, and in raw meat, poultry, eggs, and unpasteurised milk. The optimal temperature for growth of this bacteria is between 30 °C and 37 °C; therefore, it is especially important to adopt preventative measures in the warmest months of the year, which is when the risk grows considerably.

## Bibliography

AFSA, 2001. *Evaluation of microbiological methods for detection and for enumeration of microbiological contaminants in food. Final report Contract SMT4/CT96 2098. Coordination by Agence Française de Sécurité Sanitaire des Aliments. AFSSA, France, February 2001.*

ISO 6579-1:2017. *Microbiology of the food chain – Horizontal Method for detection and enumeration and Serotyping of Salmonella - Part 1: Detection of Salmonella spp.*

ISO 6887-1:2017. *Microbiology of the food chain – Preparation on test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.*



## Method

### PRE-ENRICHMENT

25 g sample + 225 ml of Buffered Peptone Water (CAT. 1402) / Incubation: 34 °C to 38 °C – 18 ± 2 h

For environmental samples of the primary production phase or of faeces, spread directly over Agar MSRV

### SELECTIVE ENRICHMENT

0.1 ml + 10 ml Rappaport Soy Broth (Vassiliadis) (CAT. 1174) or Modified Semisolid Rappaport Vassiliadis Medium (MSRV) (CAT. 1376) Incubation: 41.5 °C ± 1 °C – 24 ± 3 h

**Warning: When incubating the MSRV plate, do not invert the plate**

### SELECTIVE ENRICHMENT

1 ml + 10 ml Muller Kaufmann Broth with brilliant Green and Novobiocin (MKTTN) (CAT. 1173) Incubation: 37 °C ± 1 °C – 24 ± 3h

### SELECTIVE ISOLATION

XLD Agar (Xylose Lysine Desoxycholate Agar) (CAT. 1274) Incubation: 41.5 °C ± 1 °C – 24 ± 3 h

### SELECTIVE ISOLATION

As a secondary medium, select among the following:  
 Salmonella Shigella Agar (SS Agar) (CAT.1064)  
 Hektoen Enteric Agar (CAT. 1030)  
 Brilliant Green Agar Modified (CAT. 1143)  
 Bismuth Sulfite Agar (Wilson Blair) (CAT. 1011)  
 Salmonella Chromogenic Agar (CAT. 1122)

For result reading and suspect colony selection, refer to the technical data sheet of each product. Next, select a single suspect colony for spreading in non-selective culture medium. If the result is negative, test 4 suspect colonies previously marked

### ISOLATION IN NON-SELECTIVE AGAR

Nutrient Agar (CAT. 1060) or Nutrient Agar with Sodium Chloride (CAT. 1355) / Incubation: 34-38 °C – 24 ± 3 h

### CONFIRMATION

Complete a biochemical and serological test on the grown colonies in the non-selective agar.

The biochemical test shall consist of:

- Triple Sugar Iron Agar (TSI) (CAT. 1172)
- Christensen's Urea Agar (CAT. 2000)
- Lysine Decarboxylase Medium (CAT. 1176)
- β-galactosidase (optional)
- Indole reaction (optional)

#### NOTE:

\*According to Appendix D of ISO 6579-1:2017, the procedure for the detection of enteric subspecies (*S. typhi* and *S. paratyphi*) should be as follows:

- 1.- Selenite Cystine Broth (CAT. 1220) as selective enrichment
- 2.- As selective secondary medium, select Bismuth Sulfite Agar (Wilson Blair) (CAT. 1011)



# Detection and enumeration of *Listeria spp*

Procedure as defined by ISO 11290:2017

## Introduction

Gram-positive bacteria with basophil morphology belong to the genus *Listeria*. It is a genus composed of only 6 species, with *Listeria monocytogenes* being the most important since it is a pathogenic species present in foods suitable for human consumption.

*L. monocytogenes* causes listeriosis (food poisoning with a mortality rate close to 30%). These bacteria are gram-positive, very small in size, catalase-positive, and non-sporulating. They are facultative anaerobic, capable of growing in a wide range of temperatures (1 °C to 45 °C) and in high salt concentrations. Additionally, it is a flagellated microorganism and, as such, it is motile, which further increases its pathogenicity.

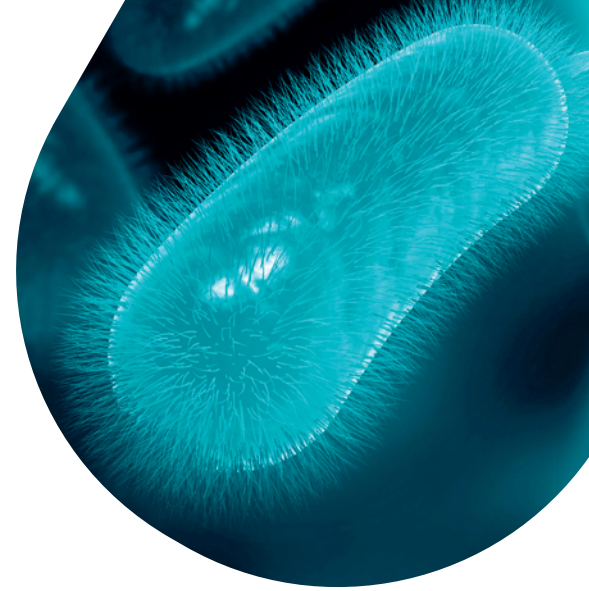
The microorganism is widely distributed in nature and it is very common to find it in food processing plants, where its survival capacity in such a wide range of temperatures makes it, perhaps, one of the key microorganisms to control in the industry.

## Bibliography

KATHARIOUS S. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. 2002; 65: 1881-1829.

ISO 11290-1. *Microbiology of the food chain – Horizontal Method for detection and enumeration of Listeria monocytogenes and Listeria spp - Part 1: Detection Method.*

ISO 11290-2. *Microbiology of the food chain – Horizontal Method for detection and enumeration of Listeria monocytogenes and Listeria spp - Part 2: Enumeration Method.*



## 1 - DETECTION METHOD (ISO 11290-1)

### PRIMARY SELECTIVE ENRICHMENT

25 g/ml sample + 225 ml of Listeria Half-Fraser Broth (CAT. 1183 + CAT. 6050)  
Incubation: 30 °C ± 1 °C – 24 + 2 h

You could then go directly to spread directly on the ALOA Selective Agar without secondary selective enrichment

### SECONDARY SELECTIVE ENRICHMENT

0.1 ml of primary culture + 10 ml of Listeria Fraser Broth (CAT. 1182 + CAT. 6050)  
Incubation: 37 °C ± 1 °C – 24 ± 2 h

### SELECTIVE ISOLATION

Listeria Chromogenic Agar according to Ottaviani and Agosti (ALOA) (CAT. 1345) and Supplements (6031 + 6040) Incubation: 37 °C ± 1 °C – 24/48 ± 2h

### SELECTIVE ISOLATION

As secondary medium, select among the following: Listeria Oxford Agar (CAT. 1133 + 6003)  
Listeria Palcam Agar (CAT. 1141 + 6004)

Reading of *Listeria monocytogenes* suspect colonies in ALOA agar: Greenish-blue colonies with opaque halo (also consider those without a halo for *Listeria spp*). For colony reading in secondary agar, temperatures and incubation times, refer to the relevant technical data sheets

### ISOLATION IN NON-SELECTIVE AGAR

Choose between: Nutrient Agar (CAT. 1060), Nutrient Agar with Sodium Chloride (CAT. 1355), Blood Agar N°2 (CAT. 1328), TSYEA Agar (Tryptone Soy Yeast Extract Agar) (CAT. 1398) or TSYEB Broth (Tryptone Soy Yeast Extract Broth) (CAT. 1339) / Incubation: According to specification. Refer to relevant technical data sheet

### L. MONOCYTOGENES CONFIRMATION

- Microscopy
- Hemolytic Activity – Blood Agar N°2 (CAT. 1328)
- Carbohydrates Utilization Broth (CAT. 1342)
- Catalase (optional)
- Motility (optional)
- CAMP test (optional)

### LISTERIA SPP CONFIRMATION

- Microscopy
- Catalase
- Motility (optional)
- Voges-Proskauer Reaction (optional)

## 2 - ENUMERATION METHOD (ISO 11290-2)

### INITIAL SUSPENSION

x g/ml sample + 9 x g/ml of diluent according to ISO 6887

In the event that the enumeration is performed on the same sample as the detection, Listeria Half-Fraser Broth (CAT. 1183) and Listeria Fraser Broth (CAT. 1182) should be used in the same proportions as indicated above

### SELECTIVE ISOLATION

Listeria Chromogenic Agar according to Listeria Chromogenic Agar according to Ottaviani and Agosti (ALOA) (CAT. 1345) and Supplements (CAT.6031 + CAT. 6040) / Incubation: 37 °C ± 1 °C

*Listeria monocytogenes* suspect colony reading in ALOA Agar: Greenish-blue with opaque halo (also consider those without halo for *Listeria spp.*)

### ISOLATION IN NON-SELECTIVE AGAR

Choose between: Blood Agar N°2 (CAT. 1328) or TSYEA Agar (Tryptone Soy Yeast Extract Agar) (CAT. 1398) / Incubation: 37 °C ± 1

### L. MONOCYTOGENES CONFIRMATION

- Microscopy
- Hemolytic Activity – Blood Agar N°2 (CAT. 1328)
- Carbohydrates Utilization Broth (CAT. 1342)
- Catalase (optional)
- Motility (optional)
- CAMP test (optional)

### LISTERIA SPP CONFIRMATION

- Microscopy
- Catalase
- Motility (optional)
- Voges-Proskauer Reaction (optional)

# Detection and enumeration of *Campylobacter* spp

Procedure as defined by ISO 10272:2017

## Introduction

Microorganisms of the genus *Campylobacter* are gram-negative bacilli with a comma or curved spiral shape, motile by means of a unipolar or bipolar flagellum and microaerophiles.

They are thermophilic bacteria with optimal growth at temperatures between 42-43 °C. Additionally, they are the most frequent cause of gastroenteritis in humans.

The World Health Organization (WHO 2011) considers that, at a global level, *Campylobacter* is one of the primary causes of food-transmitted diarrhoeal diseases. The majority of cases of campylobacteriosis are associated with food consumption, mainly poultry; therefore, these bacteria represent a top-priority in food safety.

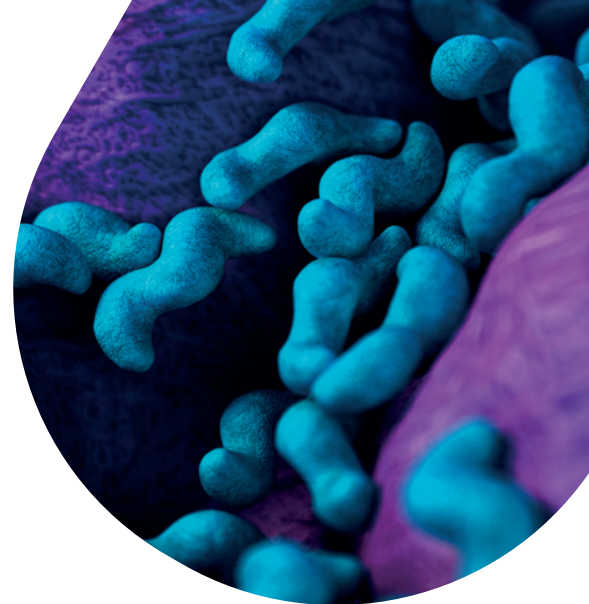
## Bibliography

AESAN (2012). *Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) con relación a las medidas de control para reducir la presencia de Campylobacter spp. en carne fresca de aves (pollo)*. Revista del Comité Científico, N° 16: 21-55.

OMS (2011) *Campylobacter*. Nota descriptiva núm. 255. Octubre de 2011.

UNE-ENISO 10272-1:2017. *Microbiology of the food chain – Horizontal Method for detection and enumeration of Campylobacter spp. Part 1: Detection Method*.

UNE-ENISO 10272-2:2017. *Microbiology of the food chain – Horizontal Method for detection and enumeration of Campylobacter spp. Part 2: Colony Count Technique*.



### 1 - DETECTION BY ENRICHMENT: Samples with low concentration of *Campylobacter* and low accompanying microflora levels (e.g. cooked or frozen products)

#### SELECTIVE ENRICHMENT

10 ml/g sample + 90 ml of Bolton Selective Enrichment Broth (CAT. 1441 + CAT. 6070) / Incubation in microaerobic atmosphere: 37 °C – 4 h / 6h then 41.5 °C – 44h ± 2 h

#### PRESUMPTIVE ISOLATION

*Campylobacter* Agar Blood Free (CCDA) (CAT. 1129 + CAT. 6053) Incubation in microaerobic atmosphere: 41.5 °C – 44h ± 2 h

In turn, spread in secondary selective medium under the same conditions as incubation. One of the ISO-compliant culture media is *Campylobacter* Agar (Preston) (CAT. 1131 + CAT. 6019)

#### RESULT READING

Typical colonies appear greyish, often showing a shiny metallic tone. They are usually flat with a humid aspect, and have a tendency to disseminate. Other morphologies could also be present

#### CONFIRMATION

Execute the following on the suspect colonies grown in CCDA Agar:

- Spreading in Columbia Agar (CAT. 1104) Incubation in microaerobic atmosphere at 41.5 °C – 24/48 h
- Examination of morphology and motility under the microscope: Motile, flagellated and curved bacilli
- Study of aerobic growth at 25 °C: Negative growth in Columbia Agar (CAT. 1104) under aerobic conditions during 44± 4 h
- Oxidase Activity (+)

## 2 - DETECTION BY ENRICHMENT: samples with low concentrations of *Campylobacter* and high accompanying microflora levels (e.g. meat or raw milk)

### SELECTIVE ENRICHMENT

10 ml/g sample + 90 ml of Campylobacter Preston Broth (CAT. 2166 + CAT. 6081) / Incubation in microaerobic atmosphere: 41.5 °C – 44 h ± 2 h

### PRESUMPTIVE ISOLATION

Campylobacter Agar Blood Free (CCDA) (CAT. 1129 + CAT. 6053) Incubation in microaerobic atmosphere: 41.5 °C – 44 h ± 2 h

### RESULT READING

Typical colonies appear greyish, often showing a shiny metallic tone. They are usually flat with a humid aspect, and have a tendency to disseminate. Other morphologies could also be present

### CONFIRMATION

Execute the following on the suspect colonies grown in CCDA Agar:

- Spreading in Columbia Agar (CAT. 1104) / Incubation in microaerobic atmosphere at 41.5 °C – 24/48 h
- Examination of morphology and motility under the microscope: Motile, flagellated and curved bacilli
- Study of aerobic growth at 25 °C: Negative growth in Columbia Agar (CAT. 1104) under aerobic conditions during 44± 4 h
- Oxidase Activity (+)

## 3 - DETECTION BY DIRECT SPREADING: Samples with a high concentrations of *Campylobacter* (e.g. faeces, raw poultry)

### PRESUMPTIVE ISOLATION

Campylobacter Agar Blood Free (CCDA) (CAT. 1129 + CAT. 6053) Incubation in microaerobic atmosphere: 41.5 °C – 44 h ± 2 h

In turn, spread in secondary selective medium under the same conditions as incubation. One of the ISO-compliant culture media is Campylobacter Agar (Preston) (CAT. 1131 + CAT. 6019)

### RESULT READING

Typical colonies appear greyish, often showing a shiny metallic tone. They are usually flat with a humid aspect, and have a tendency to disseminate. Other morphologies could also be present

### CONFIRMATION

Execute the following on the suspect colonies grown in CCDA Agar:

- Spreading in Columbia Agar (CAT. 1104) / Incubation in microaerobic atmosphere at 41.5 °C – 24/48 h
- Examination of morphology and motility under the microscope: Motile, flagellated and curved bacilli
- Study of aerobic growth at 25 °C: Negative growth in Columbia Agar (CAT. 1104) under aerobic conditions during 44± 4 h
- Oxidase Activity (+)



# Detection of *Cronobacter spp*

Procedure as defined by ISO 22964:2017

## Introduction

Bacteria belonging to the *Cronobacter* genus are gram-negative, facultative anaerobic, oxidase-negative and catalase-positive bacilli. They are usually motile and have the capacity to produce a variety of acids coming from multiple different carbohydrates. In fact, such attribute is used in the method confirmation stage.

The best known type of this genus is the *Cronobacter sakazakii*. Such type is considered nowadays an emerging pathogen that causes severe meningitis in infants. These processes may result in a percentage of mortality ranging between 40-80 %.

## Bibliography

Fiedemann M., *Enterobacter sakazakii* in food and beverage (other than infant formula and milk powder). Int. J. Food Microbiol. 2007; 116:1-10.

ISO 22964:2017. Microbiology of the food chain. Horizontal method for the detection of *Cronobacter spp*.

Center of Disease Control and Prevention. *Enterobacter sakazakii* infections associated with the use of powered infant formula. JAMA, 2002; 287:2204 - 2205.



## Method

### INITIAL SUSPENSION

- 10 ml/g sample + 90 ml of Buffered Peptone Water (CAT. 1402) / Incubation: 38 °C – 24 ± 2 h

### SELECTIVE ENRICHMENT

- 0.1 ml pre-enriched sample + 10 ml of Cronobacter Selective Broth (CSB) (CAT. 2143)  
Incubation: 41.5 °C – 24 ± 2 h

### PRESUMPTIVE SELECTIVE ISOLATION

- Chromogenic Cronobacter Isolation Agar (CCI) (CAT. 1446) / Incubation: 41.5 °C – 24 ± 2 h

### RESULT READING

- The suspect colonies of *Cronobacter sakazakii* are small in size (1-3 mm) showing a blue or greenish blue colour

The white, white with a green centre, grey or black colonies, as well as the yellow or red pigmented colonies, do not correspond to the *Cronobacter* genus type

### ISOLATION IN NON-SELECTIVE AGAR

- Streaking in Trypticasein Soy Agar (TSA) (CAT. 1068)  
Incubation: 34-38 °C - 21 ± 3 h

### BIOCHEMICAL CONFIRMATION

- Oxidase assay (-)
- Enzyme α-glucosidase assay
- Lysine Decarboxylase Broth (-) (CAT. 1208)
- L-Ornithine Decarboxylation Medium (CAT. 2149)
- Carbohydrate Fermentation Medium
- Methyl Red (optional)
- Voges-Proskauer (optional)

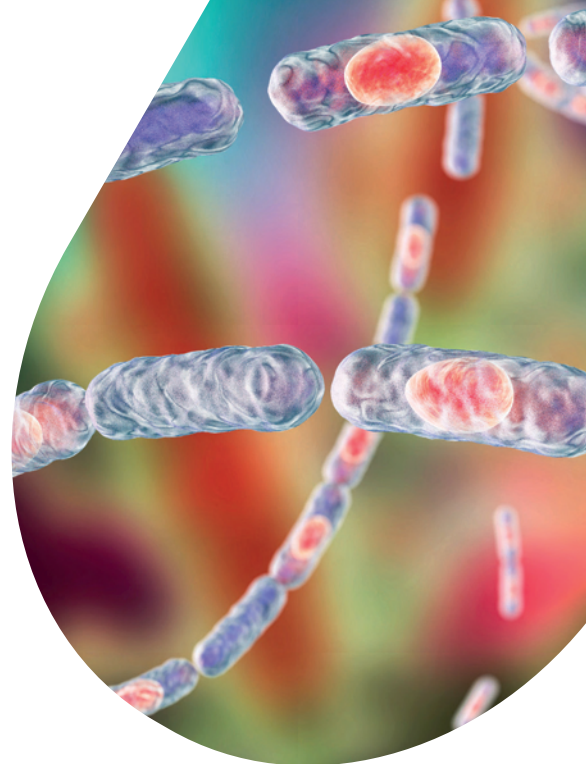
# Presumptive *Bacillus Cereus* count

Procedure as defined by ISO 7932:2004

## Introduction

*Bacillus cereus* is a facultative anaerobic, gram-positive bacillus, former of non-deforming spores. It is also catalase-positive, and fermenter of glucose and sucrose, and also of salicin and glycerol. In addition, it produces lecithinase that can be used as a marker to establish its presence.

This microorganism is widely distributed and can be found in various foods, mainly in dehydrated products such as cookies, soups, cereals, and tea. It grows in a wide range of temperatures, which makes these bacteria very resistant to the processing of food. If we add to this characteristic their spore-forming capacity, these bacteria become of critical analysis in the food industry.



## Bibliography

MOSSELL, D.A.A. KOOPMAN, M.J, JONGERIUS, E. *Enumeration of Bacillus cereus in Foods*. 1967. *Appli. Microbiol.*, 15; 650-653.

ISO 7932:2014. *Microbiology of the food chain – Horizontal method for enumeration of presumptive Bacillus cereus. Colony Count technique at 30 °C.*

ISO 6887-1:2017. *Microbiology of the food chain – Preparation on test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.*

## Method

### INITIAL SUSPENSION

Refer to the **ISO 6887-1** standard specific to the product to be analysed

### SELECTIVE ISOLATION

Bacillus Cereus Selective Agar (MYP) (**CAT. 1343**) and Supplements (**CAT. 6021 + CAT. 5152**) / Incubation: 30 °C // 18 – 48 h

The suspect colonies of the presumptive *Bacillus cereus* show a pink colour and are surrounded by a precipitate

If the plates are overgrown and well isolated colonies cannot be selected, a purification in Bacillus Cereus Selective Agar (MYP) (**CAT. 1343**) of 5 presumptive colonies should be carried out

### CONFIRMATION

Hemolytic activity – Blood Agar N°2 (**CAT. 1328**)  
Incubation: 30 °C // 24 ± 2 h

# Detection and enumeration of *Enterobacteria*

Procedure as defined by ISO 21528:2017

## Introduction

The family of the *Enterobacteriaceae* includes around 30 types of genus formed by 100 different bacterial species. This group is characterized for being gram-negative bacteria mainly with the morphology of bacilli, although cocci and pleomorphic forms can also be found. The members of this group belong to the microbiota of the intestine although they can also be isolated in other human organs, plants, and animals.

The total count of enterobacteria is used as a marker of faecal contamination and of good manufacturing practices. Therefore, it is an element that indicates the quality of processed food products. A high count of this marker would indicate a deficient manufacturing process or a possible later contamination of the end product, implying a hygienic-sanitary risk for the consumer.

## Bibliography

MOSSELL, D.A.A. *Media for Enterobacteriaceae*. 1985. Int. J. Food. Microbiol. 2:27-35

ISO 21528-1:2017. *Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 1: Detection of Enterobacteriaceae*.

ISO 21528-2:2017. *Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count technique*.



## Method

### INITIAL SUSPENSION

- 10 ml/g sample + 90 ml of Buffered Peptone Water (CAT. 1402) / Incubation: 38 °C – 18 ± 2 h

### PRESUMPTIVE SELECTIVE ISOLATION

- Violet Red Bile Agar with Glucose (VRBG) (CAT. 1092) / Incubation: 37 °C – 24 ± 2 h

### RESULT READING

- The suspect colonies of *Enterobacteriaceae* display a reddish pink or purple colour and could also show halos of precipitation

If more than one morphology of colony is present, select one of each type for a non-selective isolation in the following stage. If expected colonies do not appear, select those of whitish colour for confirmation

### ISOLATION IN NON-SELECTIVE AGAR

- Streaking in Nutrient Agar with Sodium Chloride (CAT. 1355) / Incubation: 37 °C – 24 ± 2 h

### CONFIRMATION

- Oxidase assay (-)
- Fermentation of glucose: Tubes including 1 cm of Glucose OF Medium (CAT. 2150) and creation of an anaerobic atmosphere with sterile mineral oil (+)

# Clostridium Perfringens count

Procedure as defined by ISO 7937:2004

## Introduction

*Clostridium perfringens* is an anaerobic, gram-positive bacillus, although it is also aerotolerant in particular occasions. It is also one of the bacterial pathogens more widely distributed in the atmosphere thanks to its capacity to form spores, and it is commonly found in the intestinal microflora of humans and animals.

These bacteria can be detected in a wide range of raw foods as a result of land contamination or presence of faeces. They can be found in raw meat, fish, dehydrated soups and sauces, milk, gelatine, pasta, flour, soybean, raw vegetables, and spices.

Food poisoning caused by *C. perfringens* (due to the release of endotoxins) is commonly associated with cooked meat dishes, poultry, dry or pre-cooked food, and, less frequently, vegetables. In the case of heat-resisting strains, the heat of the cooking process provides the necessary thermal shock for the activation and germination of spores. Additionally, such cooking reduces the level of oxygen providing and optimal environment for the growth of vegetative cells.

## Bibliography

ISO 7937:2004. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of Clostridium perfringens – Colony-count technique.*



## Method

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887** or **8261** standard for the product under analysis

### SELECTIVE ISOLATION

T.S.C. Agar (Tryptose Sulfite Cycloserine) (**CAT. 1029** + **CAT. 6020**) Incubation in anaerobiosis: 37 °C – 20 ± 2 h

### RESULT READING

Select a number fewer than 150 colonies for the count

### CONFIRMATION

Choose one of the two techniques described below:

#### METHOD A

Colony purification not required

- Thioglycollate Medium (**CAT. 1533**). Incubation in anaerobiosis 37 °C – 18/24h
- Lactose Sulfite Broth (**CAT. 1009**) Incubation in anaerobiosis 46 °C – 18/24h

#### METHOD B

Requires well-isolated characteristic colonies, otherwise, spread 5 characteristic colonies in Thioglycollate Medium (**CAT. 1533**)

- Nitrate Motility Medium (**CAT. 1565**) Incubation in anaerobiosis 37 °C – 24h
- Gelatin Lactose Medium (**CAT. 1526**) Incubation in anaerobiosis at 46 °C – 18/24h

### RESULT READING

Those characteristic colonies with production of gas and the presence of a black precipitate are considered positive

### RESULT READING

Non-motile colonies, intense nitrate reducers, lactose fermenters and gelatine liquefying colonies are considered positive



# Detection and count of Coagulase-Positive Staphylococci

Procedure as defined by ISO 6888:2003

## Introduction

The staphylococci are gram-positive, catalase-positive, immotile cocci that grow in conditions of aerobiosis. *Staphylococcus* genus includes about 30 species, among which *S. aureus*, *S. saprophyticus* and *S. epidermidis* stand out.

The most pathogenic of them is *Staphylococcus aureus*, which typically causes skin infections, although it can also cause pneumonia, endocarditis, and osteomyelitis. In general, they are associated with the formation of abscesses. Some strains produce toxins that cause gastroenteritis, scalded skin syndrome and toxic shock syndrome.

Another characteristic that increases the pathogenicity of the staphylococci is their capacity to coagulate blood thanks to the production of coagulases. *S. aureus* coagulase positive is among the most ubiquitous and dangerous pathogens for humans, both for its virulence and its capacity to develop resistance to antibiotics.

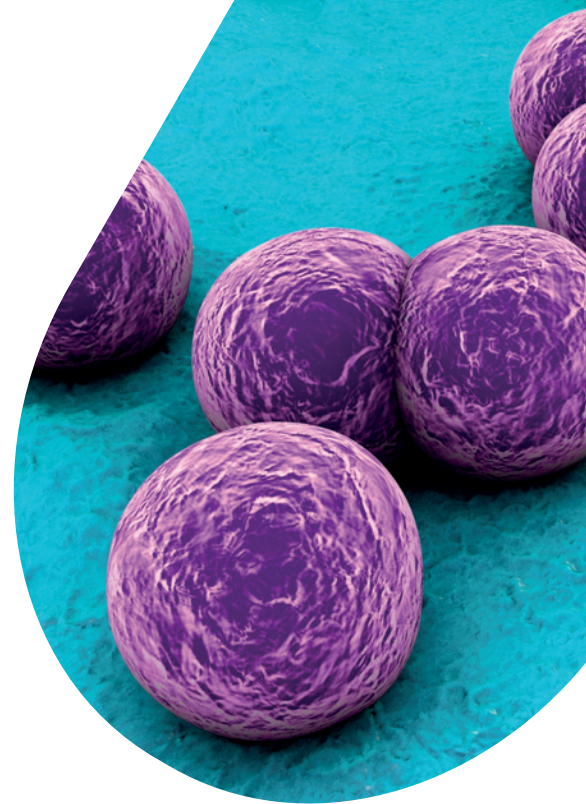
This ubiquitousness together with its virulence, makes its detection in food of utmost importance to guarantee consumer safety.

## Bibliography

ISO 6888-1:1999. *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.*

ISO 6888-2:1999/2003. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: Technique using rabbit plasma fibrinogen agar medium.*

ISO 6888-3:2003. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 3: Detection and MPN technique for low numbers.*



## ENUMERATION: PROCEDURE AS DEFINED BY ISO 6888-1

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887-1** standard specific for the product to be analysed

### PRESUMPTIVE SELECTIVE ISOLATION

Baird Parker Agar (**CAT. 1100 + CAT. 5129**) / Incubation: 35 or 37 °C – 24 ± 2 h / Reincubation after marking the characteristic colonies: 35 or 37 °C – 24 ± 2 h

### RESULT READING

The characteristic colonies are black or grey, shiny and convex, surrounded by a clear area with possible presence of an opalescent ring

### CONFIRMATION

- Spread on Brain Heart Infusion Broth (**CAT. 1331**)  
Incubation 35 or 37 °C – 24 ± 2 h
- Coagulase test (+)

## ENUMERATION: PROCEDURE AS DEFINED BY ISO 6888-2

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887-1** standard specific for the product to be analysed

### SELECTIVE ISOLATION

Baird Parker Agar (**CAT. 1319 + CAT. 6024**)  
Incubation: 35 or 37 °C – 24 ± 2 h / Reincubation after marking the characteristic colonies: 35 or 37 °C – 24 ± 2 h

### RESULT READING

The characteristic colonies are black, grey or white, surrounded by a halo of precipitation indicating coagulase activity

### CONFIRMATION

Not required, since coagulase activity is detected with RPF

## DETECTION AND MPN: PROCEDURE AS DEFINED BY ISO 6888-3

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887** or **8261** standard for the product under analysis

### ENRICHMENT

0.1 ml initial suspension +  
9 ml Giolitti-Cantoni Broth  
(**CAT. 1287**)  
Incubation: 37 °C – 24 ± 2 h

### ENRICHMENT

10 ml initial suspension + 10  
ml Giolitti-Cantoni Broth double  
concentration (**CAT. 1287**)  
Incubation: 37 °C – 24 ± 2 h

### SELECTIVE ISOLATION

Baird Parker Agar (**CAT. 1100 + CAT. 5129**) or  
Baird Parker Agar + RPF (**CAT. 1319 + CAT. 6024**)  
Incubation: 37 °C – 24 ± 2 h and 48 ± 2 h

### CONFIRMATION

The Baird-Parker method is only used for the count:

- Spread on Brain Heart Infusion Broth (**CAT. 1331**)  
Incubation 35 or 37 °C – 24 ± 2 h
- Coagulase Test (+)





# Detection of pathogenic *Yersinia enterocolitica*

Procedure as defined by ISO 10273:2017

## Introduction

*Yersinia enterocolitica* is a gram-negative, oxidase-negative, non-sporulating and facultative anaerobic type of bacteria. This type of bacteria is capable of growing in a wide range of temperatures (from -1 °C to 40 °C) and presents a capsule with antifagocytic factors, thus increasing its pathogenicity.

Its capacity to multiply in foods at low temperatures, as well as in vacuum packaged products, are the main reasons for it to be a major concern within the food industry. The majority of breakouts detected are associated with the consumption of meat, milk and unpasteurized dairy products.

Although there are many animal-derived foods that may be carriers of *Y. enterocolitica*, porcine livestock gets infected more than any other animal species.

## Bibliography

TENNANT S.H, GRANT T.H and ROBINS-BROWNE R.M. Pathogenicity of *Yersinia enterocolitica* biotype 1A. FEMS Immun. Medical Microbiol. 2003, 38 pp 127-137.

BOTTONE E.J, *Yersinia enterocolitica*. Overview and epidemiologic correlates. Microbes Infec. 1999, 1 (4) pp. 323-333.

ISO 10273:2017. Microbiology of the food chain – Horizontal method for the detection of pathogenic *Yersinia enterocolitica*.

## Method

### PRIMARY SELECTIVE ENRICHMENT

X ml/g sample + 9X ml of Sorbitol Peptone Broth and Bile Salts (PSB) (CAT. 1298)  
Incubation: 25 °C ± 1 °C – 44 ± 4h

### SECONDARY SELECTIVE ENRICHMENT

10 ml initial suspension in PSB Broth + 90 ml of Minerals Modified Glutamate Broth (MMBG) (CAT. 1365 + CAT 6051) Incubation: 25 °C ± 1 °C – 44 ± 4h

Alkaline treatment (4.5 ml KOH) during 20 ± 5S

### SELECTIVE ISOLATION

Yersinia Selective Agar (CIN) (CAT. 1126 + CAT. 6033)  
Incubation: 30 °C ± 1 °C – 24 ± 2 h

### RESULT READING

The characteristic colonies are small and circular. They present a central zone with a well-defined, dark red border. Around them, a translucent or transparent zone can be observed

### DETERMINATION OF PATHOGENIC SPECIES

- Urease test (+): (CAT. 2000)
- Bile Esculin Agar (-): (CAT.1031)
- Plasmid pYV (+)
- Detection of pyrazinamidase (-)

### BIOCHEMICAL CONFIRMATION

- Lysine Decarboxylase Broth (-): (CAT. 1208)
- Arginine dihydrolase (-)
- Phenylalanine Agar: (CAT. 1040)
- Fermentation of carbohydrates:
  - Sucrose (+)
  - Sorbitol (+)
  - Ramnosa (-)
  - Melibiosus (-)
- Simmons Citrate Agar (-): (CAT. 1014)

# Detection and enumeration of *Shigella* spp

Procedure as defined by ISO 21567:2004

## Introduction

*Shigella* is a genus of immotile, gram-negative, non-sporulating, facultative anaerobic bacillus, belonging to the *Enterobacteriaceae* family. They present reduced biochemical activity with cytochrome-oxidase negative activity and glucose fermentation without gas production.

It is a highly enteroinvasive type of bacteria: Its habitat is the colon and its principal reservoir is human beings, although it has also been isolated in other higher primates. *Shigella* species are very sensitive to changes in temperature and to unfavourable environmental conditions. However, they are able to tolerate low pH, therefore they are one of the few bacteria that can survive the acidity of the stomach and then colonize the digestive tract. This ability, combined with the fact that they are infectious in low doses, contributes to their pathogenicity.

Contamination of foods with *Shigella* may result from direct or indirect contact with faecal matter from infected persons, through infected water, pests (flies), or from lack of hygiene and proper handling techniques during food preparation.

## Bibliography

PASCUAL ANDERSON, M<sup>o</sup> R. (1992) *Microbiología Alimentaria*. Díaz de Santos, S.A. Madrid.

ATLAS, R.M., L.C. PARK (1993) *Handbook of Microbiological Media for the examination of Food*. CRC Press Inc. Boca Ratón.

ISO 21567:2004. *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Shigella spp.*



## Method

### SELECTIVE ENRICHMENT

- 1 X ml/g sample + 9X ml of Shigella Broth (CAT. 2078)  
Incubation in anaerobiosis: 41,5 ± 1 °C – 16/20 h

### SELECTIVE ISOLATION (low selectivity)

- 1 Macconkey Agar (CAT. 1052)  
Incubation: 37 ± 1 °C – 20/24 h

### SELECTIVE ISOLATION (medium selectivity)

- 2 XLD Agar (Xylose Lysine Desoxycholate Agar) (CAT. 1274)  
Incubation: 37 ± 1 °C – 20/24 h

### SELECTIVE ISOLATION (high selectivity)

- 3 Hektoen Enteric Agar (CAT. 1030)  
Incubation: 37 ± 1 °C – 20/24 h

### RESULT READING

- 1 To identify characteristic colonies of *Shigella*, refer to the relevant technical data sheets of each culture medium

### PURIFICATION COLONIES

- 1 Spread in Nutrient Agar (CAT. 1060)  
Incubation: 37 ± 1 °C – 20/24 h

### BIOCHEMICAL CONFIRMATION

- Triple Sugar Iron Agar (TSI) (Refer to reading information): (CAT. 1172)
- Semisolid Nutrient Agar (-): (CAT. 2046)
- Urea Agar (-): (CAT. 2000)
- Lysine Decarboxylase Medium (-): (CAT. 1176)
- L-ornithine decarboxylation (+/- according to spp)
- Tryptophan Culture Broth (+/- according to spp): (CAT. 1237)
- β-galactosidase Detection (+/- according to spp)
- Sugar Fermentation (according to spp)
- Acetate Differential: (CAT. 1192)

### SEROLOGICAL IDENTIFICATION

- 1
  - Antigen differentiation
  - Agglutination tests

# Detection and enumeration of *Escherichia Coli*

## Introduction

This refers to a microorganism belonging to the *Enterobacteriaceae* family. It is a gram-negative, motile, non-sporulating bacillus. Additionally, it is lactose positive and oxidase negative.

*Escherichia coli* can be distinguished from other coliforms by its ability to produce indole from tryptophan or by its production of the  $\beta$ -glucuronidase enzyme. These characteristics are used for selective isolation and the confirmation of different analytic processes as we will describe next.

Additionally, these bacteria are found in the intestines of humans and warm-blooded animals. Due to their high presence in the intestinal tract and faeces, they are considered a marker microorganism of bad hygienic practices or faecal contamination during the handling of food.

## Bibliography

ISO 7251:2005. *Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive Escherichia coli. Most probable number technique.*

ISO 16649-1:2001. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta D-glucuronide.*

ISO 16649-2:2001. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta D-glucuronide.*

ISO 16649-3:2001. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl beta D-glucuronide.*



## 1 - PROCEDURE AS DEFINED BY ISO 7251:2005 Detection and enumeration via Most Probable Number

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887** or **8261** standard for the product under analysis

### PRE-ENRICHMENT

1 ml initial suspension + 9 ml of Lauryl Sulfate Broth (Lauryl Tryptose Broth – LTB) (**CAT. 1310**) and 10 ml initial suspension + 90 ml of double concentration Lauryl Sulfate Broth. Incubation: 37 °C – 24 ± 2 h

### SELECTIVE ISOLATION

EC Medium (**CAT. 1522**)  
Incubation: 44 °C – 24/48 ± 2 h

### INDOL PRODUCTION

Inoculate tubes of Peptone Water (Tryptone Water) (**CAT. 1403**) after incubation of selective medium  
Incubation: 44 °C – 48 ± 2 h

### RESULT READING

Growth revealed by turbidity and presence of gas in EC medium and red colour in the production of indole reveal the presence of *E. coli*

**NOTE 1:** For enumeration using the MPN method (Most Probable Number), use three tubes for each dilution. In some cases, according to the matrix, 5 tubes may be required (refer to the relevant standard).

## 2 - PROCEDURE AS DEFINED BY ISO 16649-1:2001 Colony count at 44 °C using membranes

### INITIAL SUSPENSION

Refer to **ISO 6887-1** standard or the standard relevant for the product to be analysed

### SELECTIVE ENRICHMENT

On a membrane on MMG Agar plates, add 1ml of the initial suspension. Dry for 15 minutes at room temperature. / Incubation: 37 °C – 4 ± 1 h

### SELECTIVE ISOLATION

Transfer membrane to TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) (**CAT. 1151**)  
Incubación: 44 °C – 18/24 h

## 3 - PROCEDURE AS DEFINED BY ISO 16649-2:2001 Colony count at 44 °C

### INITIAL SUSPENSION

Refer to **ISO 6887-1** standard or the relevant standard for the product to be analysed

### SELECTIVE ISOLATION

TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) (**CAT. 1151**) / Incubation: 44 °C – 18/24 h

**NOTE 2:** The technician should decide between part 16649-1 and 16649-2 of the standard, keeping in mind that the first method is developed for when the sample exhibits cells with a high stress index.

## 4 - PROCEDURE AS DEFINED BY ISO 16649-3:2015 Detection and counting through the MPN technique

### INITIAL SUSPENSION

Refer to **ISO 6887-1** standard or the relevant standard for the product to be analysed

### SELECTIVE ENRICHMENT

Add a proportion of the sample/initial suspension in Minerals Modified Glutamate Broth (MMBG) (**CAT. 1365**) at single (1:9) and double (1:1) concentrations. / Incubation: 37 ± 1 °C – 24 ± 2 h

### SELECTIVE ISOLATION

Spread in TBX Chromogenic Agar (Tryptone Bile X-Glucuronide) (**CAT. 1151**)  
Incubation: 44 °C – 22 ± 2 h

**NOTE 3:** For enumeration according to the MPN (Most Probable Number) method, use 3 tubes for each dilution, which will later be used for the MPN. In some cases, according to the matrix, 5 tubes may be required (refer to the relevant standard).



# Count Total coliforms

Procedure as defined by ISO 4832:2006

## Introduction

Total coliforms are lactose-positive enterobacteria, and they constitute a group which is defined more by the tests used for their isolation than by taxonomic criteria. They belong to the *Enterobacteriaceae* family, and are characterised by their ability to ferment lactose with gas and acid production, more or less quickly, in a 48-hour period and with an incubation temperature between 30-37 °C.

They are gram-negative, facultative aerobic and anaerobic, non-sporulating bacilli. From the "coliform" group, they form various types of genus: *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, etc. They are found in the intestines of humans and animals, as well as other environments, such as water, soil, plants, egg shells, etc.

Given that it is difficult to distinguish between existing coliforms and new contaminations, it is accepted that all appearances of coliforms are new contaminations unless proven otherwise.

## Bibliography

COWELL and MORISETTI. J. Sci. Food Agric. 20, 1969, pp. 573

ISO 48321:2006. *Microbiology of food and animal feeding – Horizontal method for the detection and enumeration of coliforms – Most probable number technique*

ISO 4832:2006. *Microbiology of food and animal feeding – Horizontal method for the enumeration of coliforms – Colony-count technique*.



## Method

### INITIAL SUSPENSION

Refer to the **ISO 6887** or **8261** standard relevant to the product to be analysed

### SELECTIVE ISOLATION

1 ml of initial suspension in Violet Red Bile Agar with Lactose (VRBL) (**CAT. 1093**) Incubation: 30/37 °C – 24 ± 2 h

### RESULT READING

Select plates with fewer than 150 CFU for enumeration. The characteristic colonies have a purple colour, occasionally surrounded by a reddish halo of precipitation

### CONFIRMATION

For atypical colonies, inoculate in brilliant green bile Brilliant Green Bile Broth 2% (**CAT. 1228**). Incubation: 30/37 °C – 24 ± 2 h

# Detection of *Escherichia Coli* O157

Procedure as defined by ISO 16654:2001

## Introduction

The serotype O157 of *Escherichia coli* is rod-shaped and gram-negative. The "O" in the name refers to the antigen present in the cell wall (somatic antigen) that these bacteria present. It is an enterohaemorrhagic strain and causes food poisoning due to the production of a cytotoxic enterotoxin called verotoxin.

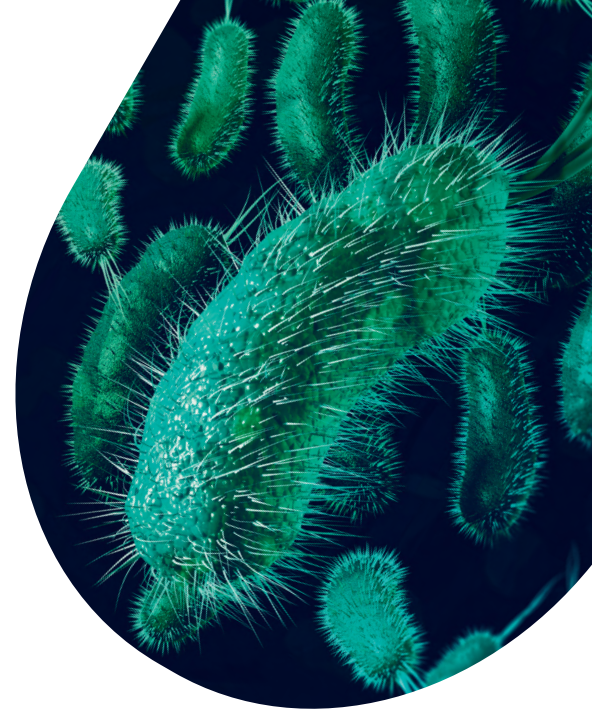
The highest incidence of the illness has been associated with the consumption of undercooked ground beef. Interpersonal contact within the family and in nursery schools is also a significant route of transmission. The infection can also occur after drinking raw milk and after swimming in or drinking water contaminated by contact with animal excrement, faecal water or sewage.

## Bibliography

ZADIK P.M, CHAPMAN P.A and SIDDONSON C.A. *J. Med. Microbiol.*, 39, 1993, pp. 155-158

DOYLE M.P. and SCHOENI J.L. *Appl. Environ. Microbiol.*, 53, 1987, pp 2394-2396

ISO 16654:2001. *Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Escherichia coli O157*



## Method

### ENRICHMENT

X g/ml sample + 9Xml of Trypticasein Soy Broth Modified with Novobiocin (mTSB) (CAT. 1292). Homogenisation and incubation 6 h and then 41.5 °C – 12/18 h

### IMMUNOMAGNETIC SEPARATION (IMS)

For 6 hours and later, after 12/18 h of incubation

### SELECTIVE ISOLATION

50µl of re-suspended magnetic particles in Macconkey Agar with Sorbitol (CF-SMAC) (CAT. 1099 + CAT. 6064) Incubation: 37 °C – 18/24 h

### SELECTIVE ISOLATION

50µl of re-suspended magnetic particles in chromogenic *E. coli* O157:H7 Chromogenic Agar (CAT. 1588 + CAT. 6064) Incubation: 37 °C – 18/24 h

### PURIFICATION COLONIES

Spread in Nutrient Agar (CAT. 1060) Incubation: 37 °C – 18/24 h

### BIOCHEMICAL CONFIRMATION

Tryptophan Culture Broth (CAT. 1237) + Kovac's Reagent (CAT. 5205)

### SEROLOGICAL IDENTIFICATION

Only for those colonies which are indole-positive



# Detection of *Vibrio* spp

Procedure as defined by ISO 21872:2007

## Introduction

Bacteria from the *Vibrio* genus are gram-negative bacilli, with comma-shaped cells. *Vibrio* is oxidase positive, facultative anaerobic, and does not form spores. All species from the genus are motile, generally with a unique polar flagellum.

Various *Vibrio* species are pathogenic, causing digestive tract illnesses, especially *V. cholerae*, the agent which causes cholera; *V. parahaemolyticus* which causes self-limiting inflammatory diarrhea, and *V. vulnificus*, which is transmitted by the intake of shellfish.

*Vibrio* infection originates in most cases by consumption of raw or undercooked shellfish. These bacteria naturally grows in marine environments, whether in salt water or estuaries, where there is a mix of fresh and salt water. This aquatic presence makes the fishing products the foods more related with the species in the *Vibrio* genus.

## Bibliography

ISO 21872-1. *Microbiology of the food chain – Horizontal method for the Vibrio spp. – Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.*



## Method

### PRIMARY ENRICHMENT

25 g/ml sample + 225 ml of Buffered Peptone Water (CAT. 2155)  
Incubation (fresh product): 41.5 °C ± 1 – 6 ± 1 h  
Incubation (others): 37 °C ± 1 – 6 ± 1 h

### SECONDARY ENRICHMENT

1 ml sample + 10 ml Buffered Peptone Water (CAT. 2155) / Incubation (*V. cholerae* and *V. parahemolyticus*) 41.5 °C ± 1 – 6 ± 1 h.  
Incubation (*V. vulnificus*) 37 °C ± 1 – 6 ± 1 h

### SELECTIVE ISOLATION

1 µl of culture incubated in TCBS Agar (CAT. 1074)  
Incubation: 37 °C ± 1 – 24 h ± 3 h

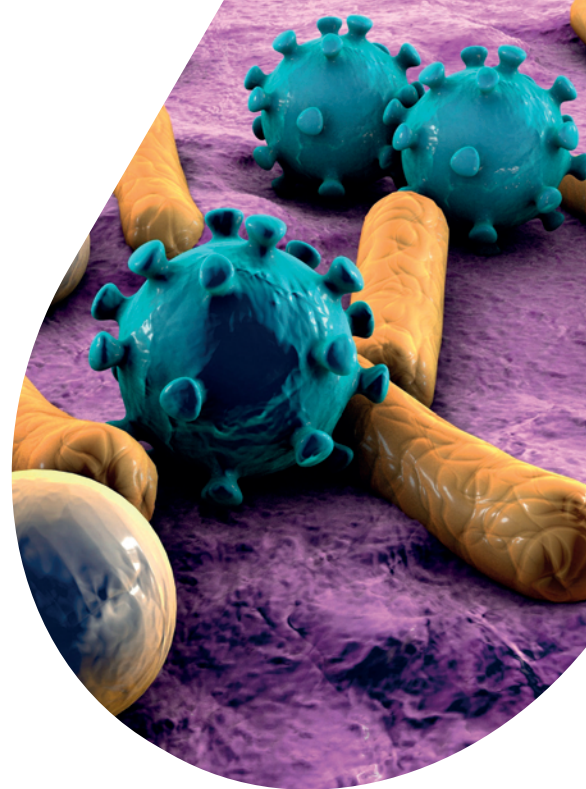
The standard also includes a secondary selective medium to be selected by the laboratory

### BIOCHEMICAL CONFIRMATION

- Decarboxylase L-Lysine in saline medium
- Arginine Dihydrolase in saline medium
- β-galactosidase detection
- Indole detection
- Halotolerance

For result reading, refer to the relevant standard

# Detection and enumeration of other microorganisms in the food industry



## SULPHITE REDUCING BACTERIA COUNT IN ANAEROBIC CONDITIONS (ISO 15213:2003)

### INITIAL SUSPENSION

Refer to **ISO 6887** or **8261** standards relevant according to the product to be analysed (proportion 1:9)

### SELECTIVE ISOLATION

1 ml initial dilution on Iron Sulfite Agar (**CAT. 1559**)  
Incubation for thermophilic bacteria: 50 °C ± 1 – 24/48 h  
Incubation for mesophilic bacteria: 37 °C ± 1 – 24/48 h

### BIOCHEMICAL CONFIRMATION

- Breathing Test
- Sporulation Test

## LACTIC BACTERIA COUNT (ISO 15214:1998)

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887-1** standard specific for the product to be analysed

### SELECTIVE ISOLATION

1 ml initial dilution on MRS Agar Low pH (**CAT. 1433**)  
Incubation: 30 °C - 72 h ± 3 h

## Bibliography

ISO 15213:2003. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.*

ISO 13720:2010. *Meat and meat products. Enumeration of presumptive Pseudomonas spp.*

ISO 6611:2004//IDF 94. *Milk and milk products – Enumeration of colony-forming units of yeast and/or molds – Colony-count technique at 25 °C.*

## MOLD AND YEAST COUNT IN DAIRY PRODUCTS (ISO 6611:2004)

### INITIAL SUSPENSION

Refer to **ISO 8261** standard relevant to the product to be analysed

### SELECTIVE ISOLATION

1 ml of initial dilution on OGA Medium (Oxytetracycline Glucose Agar) (OGYE) (**CAT. 1527 + CAT. 6018**)  
Incubation: 25 °C ± 1 – 5 days

The standard also includes the Chloramphenicol Agar (YGC Agar) (**CAT. 1301**) as a medium for selective isolation

## ALLEGED PSEUDOMONAS SPP IN MEAT AND MEAT PRODUCTS (ISO 15213:2003)

### INITIAL SUSPENSION

Refer to the relevant **ISO 6887** or **8261** standard for the product under analysis

### SELECTIVE ISOLATION

0,1 ml initial dilution on Pseudomonas CFC Agar (**CAT. 1356 + CAT. 6036**)  
Incubation: 25 °C ± 1 – 44 h ± 4 h

### BIOCHEMICAL CONFIRMATION

- Oxidase test (+)



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