Bile Esculin Azide Agar ISO

Cat. 1005

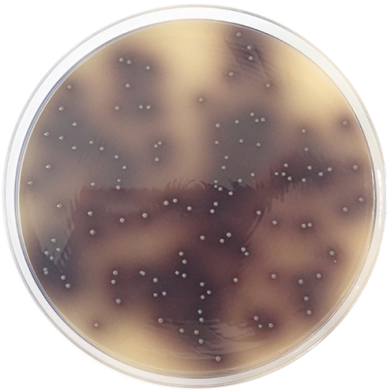
For the selective isolation and presumptive identification of intestinal enterococci by membrane filtration method

Practical information

Aplications	Categories
Selective isolation	Enterococci

Industry: Water

Regulations: ISO 7899-2



Principles and uses

Bile Esculin Azide Agar is a modification of Bile Esculin Agar (Cat. 1031), with the addition of sodium azide as an inhibitor and with the reduction of the bile concentration. The resulting medium is more selective but still provides rapid growth and efficient recovery of enterococci. The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci. Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric amonium citrate to form a dark brown or black colony. Ox bile does not inhibit enterococci while other Gram positive bacteria are inhibited. Sodium azide inhibits Gram negative bacteria. Tryptone, peptone and yeast extract supply the nutrients essential for growth. Sodium chloride provides the osmotic balance. Bacteriological agar is the solidifying agent.

The presence of intestinal enterococci is an indicator for faecal contamination, especially when the contamination occurred a long before and the less resistant coliform bacteria, including Escherichia coli, may already be dead when the analysis is carried out.

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of enterococci. Appears a brown color (positive reaction) around the colonies.

Formula in g/L

Bacteriological agar	15	Esculin	1
Ferric ammonium citrate	0,5	Ox Bile	10
Peptone	3	Sodium azide	0,15
Sodium chloride	5	Tryptone	17
Yeast extract	5		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 56,6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 50-60 ºC and dispense into appropriate containers. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position.

Instructions for use

For the detection and enumeration of enterococci according to ISO 7899-2:

- Filter a measured volume of water through a membrane filter.
- Place the membrane on a Slanetz-Bartley Medium (Cat. 1109).

- Incubate at 36±2 ºC for 44±4 h.
- Transfer the membrane with characteristic colonies previously incubated in the Slanetz-Bartley medium (Cat. 1109), without inverting the membrane, to a plate with Bile Esculin Azide Agar, pre-heated to 44ºC.
- Incubate at 44±0,5 ºC for 2 hours.
- Read the plate immediately.
- It is considered that the typical colonies that show a brown-black color in the surrounding medium give positive reactions and are recounted as intestinal enterococci.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Toasted	Litmus	7,1 ± 0,1

Microbiological test

Incubation conditions: (44±0,5 °C / 2 h).
Inoculation conditions: Confirmation (transference from membrane previously incubated in Slanez&Bartley medium [E. faecalis and E. faecium] / TSA [E. coli]).

Microorganisms	Characteristic reaction
Enterococcus faecalis ATCC 19433	Brown-black halo
Escherichia coli ATCC 25922	Absence of brown-black halo
Enterococcus faecalis ATCC 29212	Brown-black halo
Enterococcus faecium ATCC 6057	Brown-black halo

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 7899-2. Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method.
Facklam, R.R. and M.D. Moody 1970. Presumptive identification of Group D Streptococci: The bile-esculin test. Appl. Microbiol 20:245.
Ruoff, K.L. 1995 Streptococcus. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (eds), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Listeria Agar Base Palcam ISO

Cat. 1141

Selective and differential medium for the detection of Listeria spp, particularly Listeria monocytogenes.

Practical information

Aplications	Categories
Selective isolation	Listeria

Industry: Clinical / Food

Regulations: ISO 11290

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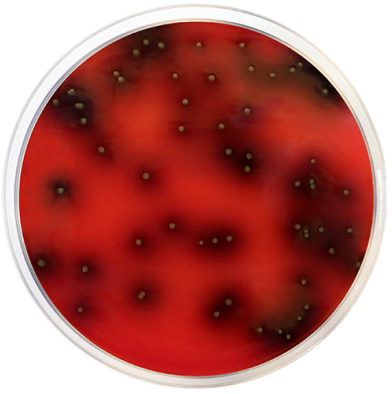


Principles and uses

Listeria Agar Base Palcam, used with supplements, is a selective and differential medium for Listeria spp. It is recommended by ISO 11290 for the detection and enumeration of Listeria monocytogenes in food products and clinical samples, and can also be used for environmental samples.

It is used after a primary and secondary enrichment stage, using Listeria Enrichment Broth Base (Cat.1120). It allows the easy differential diagnosis of Listeria monocytogenes using a double-system indicator: Esculin/Iron and Mannitol/Phenol red. All Listeria species hydrolyze the esculin to esculetin, which reacts with iron ions producing a blackening of the medium.

Lithium chloride included in the medium, along with ceftazidime, polymyxin B sulfate and Acryflavine from the supplement, inhibit the growth of the non-Listeria accompanying bacteria present in foods, which can hydrolyze the esculin. Peptones and maize starch provide a rich nutrient base for growth. Yeast extract is the source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate. Ferric ammonium citrate improves the growth of L. monocytogenes.

The Mannitol/Phenol red differentiation system is used to differentiate Listeria spp that do not ferment mannitol from other species that occasionally grow in the medium such as enterococci or staphylococci. Differentiation is achieved by the acid increase in the media, causing the phenol red indicator to change the color of the medium from red to yellow. Confirmation of Listeria is done by biochemical and serological identifications tests.

Formula in g/L

Glucose	0,5	Bacteriological agar	10
Esculin	0,8	Ferric ammonium citrate	0,5
Maize starch	1	Mannitol	10
Peptone	23	Phenol red	0,08
Sodium chloride	5	Yeast extract	3
Lithium chloride	15		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 34,4 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC and aseptically add one vial of Palcam Listeria Selective Supplement (Cat. 6004). Homogenize gently and dispense into Petri dishes.

Instructions for use

For clinical diagnosis, the type of sample is amniotic fluid.
- Inoculate on the surface making parallel striae with the handle or swab.

- Incubate in aerobic conditions at 35±2 ºC for 24-48 hours.
- Reading and interpretation of the results.

For the detection and enumeration of Listeria monocytogenes and Listeria spp. according to ISO 11290:
Primary enrichment:
- Weigh 25 g (or 25 ml) of the sample and add 225 ml of Listeria 1/2 Fraser Broth (Cat. 1120 + Cat. 6002). Homogenize and incubate at 30 ºC for 25±1 h.
Secondary enrichment:
- Inoculate 0,1 ml of the culture of the Listeria 1/2 Fraser Broth incubated (regardless of its color) in 10 ml of Listeria Fraser Broth (Cat. 1120 + Cat. 6001).
Incubate at 37 ºC for 24±2 hours under aerobic conditions.

Plaque and identification:
- From the primary enrichment culture, the Listeria Agar surface is inoculated according to Ottaviani and Agosti (Cat. 1345), to obtain well separated colonies.
- From the secondary enrichment culture, the procedure is repeated, inoculate the surface of the Listeria Agar according to Ottaviani and Agosti, the Palcam Listeria Agar (Cat. 1141) and another medium such as the Oxford Agar (Cat. 1133).
- For Listeria Agar according to Ottaviani and Agosti incubate for a total of 48±2 h.
- For Agar Lisetria Palcam incubate at 35±2 ºC for 24-48 h.
- For Oxford agar incubate at 35±2 ºC for 24-48 h.

Confirmation:
- Select the presumptive colonies and carry out confirmatory tests for L. monocytogenes or Listeria spp.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Red	7,2±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (35±2 ºC / 24-48 h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA.

Microorganisms	Specification	Characteristic reaction
Listeria monocytogenes 4b ATCC 13932	Good growth (2)	Green-gray colonies with black center and black halo.
Escherichia coli ATCC 25922	Total inhibition (0)	
Enterococcus faecalis ATCC 29212	Total inhibition (0)	
Listeria monocytogenes ATCC 7644	Good growth (2)	Green-gray colonies with black center and black halo

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO NORMATIVE 11290-2:Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 2: Enumeration method.
Van Netten, P., I. Perales A. Van de Moosalijk G.D.W. Curtis and DAA Mossel 1989 Liquid and solid selective differential media for the detection and enumeration of L. monocytogenes and other Listeria spp. Int. J. of Food Microbiol 8: 299-317.
Farber JMDW Warburton and T. Babiuk, 1994 Isolation of Listeria monocytogenes from all food and environmental samples.

Rose Bengal Agar + Cholamphenicol + Dichloran (DRBC Agar) ISO

Cat. 1160

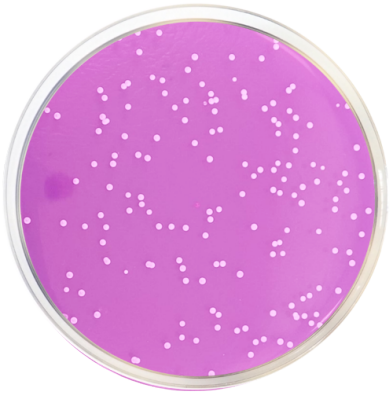
Selective medium for the enumeration of yeasts and molds in foods.

Practical information

Aplications	Categories
Selective isolation	Yeasts and molds

Industry: Food / Dairy products

Regulations: ISO 11133 / ISO 21527



Principles and uses

Rose Bengal Agar + Cholamphenicol + Dichloran (DRBC Agar) is a selective medium recommended by ISO 21527-1 for the enumeration of yeasts and molds, by means of the colony count technique, in foods products for human consumption and animals feeding stuffs which have a water activity greater than 0,95, such as meat, eggs, dairy products (except milk powder), fruits, fresh pastes, vegetables, etc. This formula is a modification of Rose Bengal Agar.

Peptone provides the nitrogen, vitamins, minerals and amino acids source. Dextrose is the fermentable carbohydrate as a carbon and energy source. Potassium phosphate is the buffer. Magnesium sulfate provides sulfur and other trace elements. Rose bengal is a selective agent that inhibits the growth of bacteria and limits the size and height of faster-growing molds, allowing for the development and detection of other slower-growing yeasts. Molds appear pink colored. Chloramphenicol serves as a selective agent, inhibiting bacterial growth. It is a recommended antibiotic for neutral media due to its heat stability and wide bacterial spectrum. The addition of dichloran prevents the fast spreading of mucoraceous fungi and also restricts the size of the colonies of other genera, improving the colony count. Bacteriological agar is the solidifying agent.

Formula in g/L

Glucose	10	Bacteriological agar	15
Chloramphenicol	0,1	Monopotassium phosphate	1
Rose bengal	0,025	Dichloran	0,002
Enzymatic Digest of Plants & Animal Tissue	5	Magnesium sulfate monohydrate	0,5

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 31,6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 44-47 ºC, mix well and dispense into plates.

Instructions for use

For the enumeration of yeasts and moulds according to ISO 21527:
- Prepare the test portion, initial suspension (primary dilution) and further dilutions.
- Transfer 0,1 ml of the test sample to one DRBC agar plate.
- On a second DRBC agar plate, transfer 0,1 ml of the first decimal dilution (10^-1), or 0,1 ml of the 10^-2 dilution.
- Spread the liquid over the surface of the agar plate until the liquid is completely absorbed into the medium.
- Incubate the prepared plates aerobically, lids uppermost at 25±1 ºC for 5 days.
- Count and select the colonies for confirmation.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Pink	Intense pink	5,6±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (25±1 ºC / 5 days).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU).
Reference medium: SDA

Microorganisms	Specification	Characteristic reaction
Candida albicans ATCC 10231	Good growth (2) >50%	Characteristic colony/propagules according to each species.
Aspergillus brasiliensis ATCC 16404	Good growth (2) >50%	Characteristic colony/propagules according to each species.
Escherichia coli ATCC 25922	total inhibition (0)	
Mucor racemosus ATCC 42647	Good growth (2) >50%	Characteristic colony/propagules according to each species.
Bacillus subtilis ATCC 6633	Total inhibition (0)	
Saccharomyces cerevisiae ATCC 9763	Goog growth (2) >50%	Characteristic colony/propagules according to each species.

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

King; D.A. and Pitt, J.I(1 979) Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. Appl. Environm. Microbiol. 37 959-964
ISO 21527 - Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0,95.

Dichloran Glycerol Agar (DG 18) ISO

Cat. 1161

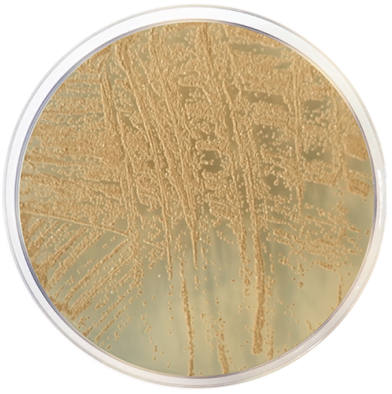
For the enumeration and isolation of xerophilic fungi in dry and semi-dry foods

Practical information

Aplications	Categories
Selective enumeration	Xerophilic fungi
Selective isolation	Xerophilic fungi

Industry: Food / Dairy products

Regulations: ISO 11133 / ISO 21527



Principles and uses

Dichloran Glycerol Agar (DG 18) is a selective medium based on the formulation of Hocking and Pitt. It is recommended for the enumeration and isolation of xerophilic molds from dried and semi-dried foods, such as fruits, spices, cereals, nuts, meat and fish products.

Glycerol reduces the water activity from 0.999 to 0.95, thereby reducing bacterial growth, and is also the carbon source.
Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is a recommended antibiotic for use with media due to its heat stability and wide
bacterial spectrum. Bacteria growth inhibition and spreading of more-rapidly growing molds restriction aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. Dichloran prevents the fast spreading of mucoraceous fungi, improving the colony count. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphate acts as a buffer system. Magnesium sulfate provides sulfur and other trace elements. Bacteriological agar isthe solidifying agent.

Formula in g/L

Enzymatic digest of casein	5	Bacteriological agar	15
Chloramphenicol	0,1	D-Glucose	10
Magnesium sulfate	0,5	Potassium dihydrogenphosphate	1
Dichloran	0,002		

Preparation

Suspend 31,6 grams of the medium in one liter of distilled water. Add 175 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute and sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50ºC and pour into Petri dishes.

Instructions for use

- Use two different plates.
- On to one DG 18 plate, transfer 0,1 ml of the test sample if the sample in liquid, or 0,1 ml of the initial suspension if the sample is not liquid.
- On to second DG 18 plate, transfer 0,1 ml of the first decimal dilution (10-1) if liquid, or 0,1 ml of the second decimal dilution (10-2) if not.
- Inoculate and incubate at 25±1 °C.
- Examine for growth after 5-7 days. If the presence of Xeromyces bisporus is suspected, incubate the plates for 10 days.
- Select the dishes containing < 150 colonies and count them. If fast growing molds are a problem, count colonies after 2 days and again after 5-7 days of incubation.
- This number can be reported as number of xerophilic colonies per gram of food.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	5.6 ± 0.2

Microbiological test

According to ISO 11133:
Incubation conditions: (25±1 ºC / 5 days).
Inoculation conditions: (100±20. Min.50 cfu)/ Selectivity (10^4-10^6 cfu).
Reference media: SDA.

Microorganisms	Specification	Characteristic reaction
Escherichia coli ATCC 25922	No growth	
Wallemia sebi ATCC 42964	Good growth >50%	Characteristic colony/propagules according to each species
Bacillus subtilis ATCC 6633	No growth	
Saccharomyces cerevisiae ATCC 9763	Good growth >50 %	Characteristic colony/propagules according to each species

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 21527-2: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 2: Colony count technique in products with water activity less than or equal to 0.95

Hocking, A.D.,and Pitt,J.L. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Enviornm. Microbiol 39, 488-492

Giolitti-Cantoni Broth ISO

Cat. 1287

Liquid medium for the enumeration in accordance to the MPN method and selective enrichment of Staphylococcus aureus.

Practical information

Aplications	Categories
Selective enumeration	Staphylococcus aureus
Selective enrichment	Staphylococcus aureus

Industry: Food

Regulations: ISO 11133 / ISO 6888

Principles and uses

Giolitti-Cantoni Broth ISO is a modified formula of a medium formulated by Giolitti and Canton in 1996. It is recommended by ISO 6888-3 for the enumeration and detection of coagulase-positive staphylococci from food and animal feeding stuffs, using the MPN method..

Casein peptone and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Mannitol is the fermentable carbohydrate providing carbon and energy. Lithium chloride inhibits the growth of Gram-negative bacteria. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene and formalin. The growth of staphylococci is encouraged by sodium pyruvate and glycine. Gram-negative contaminants are inhibited by potassium tellurite.

This method is recommended for products where staphylococci are expected to be stressed and in low numbers such as dried products. Coagulase-positive staphylococci will mostly be Staphylococcus aureus, but Staphylococcus intermedius and some strains of Staphylococcus hyicus are also coagulase-positive.

The confirmation of staphylococci which produce coagulase is based on a strongly positive coagulase reaction, but it is also known that some strains of coagulase-positive staphylococci give weak positive coagulase reactions. These latter strains can be confused with other bacteria but can be differentiated by the use of additional tests such as one for the production of thermonuclease.

Formula in g/L

Beef extract	5	Casein peptone	10
Glycine	1,2	Mannitol	20
Polysorbate 80	1	Sodium chloride	5
Sodium pyruvate	3	Yeast extract	5
Lithium chloride	5		

Preparation

Suspend 55,2 grams of the medium in one liter of distilled water for the preparation of single strenght broth. Suspend 110,4 grams of the medium in one liter of distilled water for the preparation of double strenght broth. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute 9 ml portions into tubes for the preparation of single strenght broth and 10 ml for the preparation of double strenght broth. Sterilize in an autoclave at 121ºC for 15 minutes. Cool to 45-50 ° C and aseptically add 0.1 and 0.2 ml per tube, of 1% potassium tellurite solution for single and double concentration respectively.

Instructions for use

For the enumeration of coagulase-positive staphylococci according to ISO 6888-3:
- Inoculate a specified quantity of the test portion or the initial suspension for the detection method or serial dilutions for the enumeration method, in a selective culture medium (Giolitti-Cantoni Broth).
- Add 1 ml of the initial suspension to 9 ml of single-strength Giolitti Cantoni broth
- Add 10 ml of the initial suspension to 10 ml of double-strength Giolitti
Cantoni broth.
- For larger volumes of test portions, prepare the initial suspension by adding x ml or x g of test portion to 9x ml of the diluent. Then add the entire initial

suspension to 90x ml of single-strength Giolitti Cantoni broth, previously deaerated and with potassium tellurite added.
- Incubate the tubes at 37 ºC anaerobically for 24-48 hours. (Carefully pour a plug of agar or paraffin, cooled to between 44 °C and 47 °C, onto the top of the medium and allow it to solidify to form a seal).
- The presence of presumptive coagulase-positive staphylococci is indicated by the reduction of potassium tellurite (blackening or black precipitated).
- Subcultivate the presumptive positive tubes in plates of Baird Parker Agar (Cat. 1319), and incubate at 37 ºC for 24-48 hours.
- The presence of presumptive coagulase positive staphylococci is indicated by the reduction of potassium tellurite and egg emulsion.
- Confirm the typical and/or atypical colonies by a coagulase reaction.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Toasted	Amber	6,9 ± 0,2

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity (37±1 ºC /24±2 - 48±2 h) / Selectivity (37±1 ºC / 48±2 h)
Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10^4-10^6 CFU).

Microrganisms	Specification	Characteristic reaction
Staphylococcus aureus ATCC 25923 + Escherichia coli ATCC 25922	>10 colonies on Baird Parker or RPFA	Characteristic colonies according to each medium
Staphylococcus aureus ATCC 6538 + Escherichia coli ATCC 25922	>10 colonies on Baird Parker or RPFA	Characteristic colonies according to each medium
Escherichia coli ATCC 25922	Total inhibition	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

International Standard ISO 6888-3 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase
– positive staphylococci (Staphylococcus aureus and other species) Part3: Detection and MPN technique for low numbers.

Listeria Chromogenic Agar Base according to Ottaviani and Agosti (ALOA) ISO

Cat. 1345

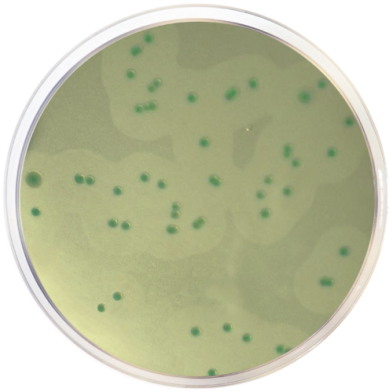
Selective medium for the detection and enumeration of Listeria monocytogenes.

Practical information

Aplications	Categories
Selective enumeration	Listeria
Detection	Listeria

Industry: Food

Regulations: ISO 11133 / ISO 11290 / BAM



Principles and uses

Listeria Chromogenic Agar Base acc. to Ottaviani and Agosti (ALOA) is a selective medium for the presumptive isolation and identification of Listeria monocytogenes and Listeria spp. in food. It is used for confirmation after using Listeria Enrichment Broth Base Fraser (Cat. 1120). This medium is also recommended by ISO 11290-1 for the detection and enumeration for Listeria monocytogenes.

Enzymatic digest of animal tissues and enzymatic digest of casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate is a source of energy for bacterial metabolism and aids in the resuscitation of stressed organisms. Glucose is the fermentable carbohydrate providing carbon and energy. Magnesium glycerophosphate is a buffering compound. Magnesium sulphate is a magnesium ion required for a large variety of enzymatic reactions, including DNA replication. The differential activity of the medium is due to two factors. Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Bacteriological agar is the solidifying agent.

The presence of the chromogenic component X-glucoside, a substrate for the detection of the enzyme ß-glucosidase, is common to all Listeria species giving the colonies their blue colour. Other organisms that possess this enzyme, for example, Enterococci, are inhibited by the selective agents within the medium and by the selective supplement. The differential activity is also obtained by lipase C substrate, upon which the specific enzyme for L. monocytogenes acts. The lipase is responsible for the opaque white halo which surrounds L. monocytogenes.

The combination of both substrates allows us to differentiate the colonies of Listeria monocytogenes from the rest of Listeria spp. since, although all are blue in colour, L. monocytogenes present an opaque white halo surrounding them.

It has been observed that some strains of Listeria ivanovii, mostly pathogenic to animals although some have caused infections in humans, also possess lipase activity.

Formula in g/L

Enzymatic digest of casein	6	Glucose	2
Bacteriological agar	13,5	Magnesium sulfate	0,5
Sodium chloride	5	Sodium hydrogen phosphate	2,5
Sodium pyruvate	2	Yeast extract	10
Enzymatic digest of animal tissues	18	Lithium chloride	10
Magnesium glycerophosphate	1	5-Bromo-4-chloro-3-indolyl-ß-D-glucopyranoside	0,05

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 35.275 grams of the medium in 470 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until

complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. To prepare more quantity of 500 ml, it is recommended to sterilize at 115 ºC for 10 minutes. Cool to 47-50 ºC and aseptically add one bottle of Listeria Lipase C Supplement (24 ml) (Cat. 6031) and one vial of Listeria Chromogenic Selective Supplement (Cat. 6040). Homogenize gently and dispense into Petri dishes.

Instructions for use

Dection and enumeration of Listeria monocytogenes and Listeria spp. according to ISO 11290:

Detection method:
- Weigh 25 g (or 25 ml) of the sample and add 225 ml of Half Fraser Broth (Cat.1183). Homogenize and incubate at 30 °C for 25±1 hours.
- Inoculate 0,1 ml of incubated Half Fraser Broth culture (regardless of its colour) into 10 ml of Fraser Broth (Cat.1182).
Incubate at 37 ºC for 24±2 hours in aerobic conditions.
- From the primary enrichment culture inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other selective medium at the choice of the laboratory, to obtain well-separated colonies.
From the secondary enrichment culture, repeat the procedure, inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other selective medium.
For Agar Listeria according to Ottaviani and Agosti incubate for a total of 48±2 h.
- Select the presumptive colonies and carry out the confirmation tests for L. monocytogenes or Listeria spp.

Enumeration method:
- Prepare an initial suspension 1:10 of sample and Buffered Peptone Water for analysis. Listeria 1/2 Fraser Broth (Cat. 1183) can be used as a diluent if the detection and enumeration procedures are carried out simultaneously.
- Inoculate 0,1 ml on the surface of Listeria Chromogenic Agar according to Ottaviani and Agosti.
- Incubate at 37 ºC for 24 ± 2 h. Incubate for an additional 24 hours in case no microbial growth is detected.
- Select the presumptive colonies and carry out confirmation tests for L. monocytogenes or Listeria spp.
- Calculate from the confirmed colonies the number of L. monocytogenes or Listeria spp colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber slightly opalescent	7,2 ± 0,2

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity, Selectivity and Specificity (37±1 ºC / 48±4 h).
Inoculation conditions: Productivity quantitative (100±20. Min.50 CFU) / Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA

Microorganisms	Specification	Characteristic reaction
Listeria monocytogenes 4b ATCC 13932	Good growth (2) >50%	Blue green colonies with opaque halo
Enterococcus faecalis ATCC 29212	Total inhibition (0)	
Listeria innocua ATCC 33090		Blue green colonies without opaque halo
Listeria monocytogenes 1/2a ATCC 35152	Good growth (2) >50%	Blue green colonies with opaque halo
Escherichia coli ATCC 8739	Total inhibition (0)	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June.
ISO 11290 Horizontal method for the detection and enumeration of Listeria monocytogenes.

TSYEA Agar (Tryptone Soy Yeast Extract Agar) ISO

Cat. 1398

For the confirmation of Listeria spp.

Practical information

Aplications	Categories
Confirmation	Listeria

Industry: Food

Regulations: ISO 11133 / ISO 11290

Principles and uses

TSYE Agar (Tryptone Soy Yeast Extract) is a general purpose medium which supports the growth of a wide variety of microorganisms.

The formula conforms to ISO 11290 and is used for the confirmation of Listeria monocytogenes colonies and to subculture suspected Listeria colonies.

Enzymatic digest of casein, yeast extract and papaic digest of soyabean meal provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium hydrogen phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

Formula in g/L

Enzymatic digest of casein	17	Glucose	2,5
Bacteriological agar	15	Papainic digest of soy bean	3
Sodium chloride	5	Yeast extract	6
Dipotassium hydrogen phosphate	2,5		

Preparation

Suspend 51 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC, mix well and dispense into plates.

Instructions for use

Confirmation of Listeria spp. according to ISO 11290:
- Select a presumptive colony of Listeria spp.
- Streak it onto the surface plate of non-selective agar TSYEA in a manner which allows the development of well-isolated colonies.
- Incubate the plates at 37 ºC for 18-24 hours.
- Typical colonies of Listeria spp. are 1 to 2 mm in diameter, convex, colorless and opaque with entire edge. When the light impacts (45º) a colony, exhibits a blue-grey color and granular surface.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7,3 ± 0,2

Microbiological test

Incubation conditions: (37 ºC / 24 h)
Inoculation conditions: Productivity qualitative (10^3-10^4 CFU).

Microrganisms	Specification
Listeria monocytogenes 4b ATCC 13932	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 11290. Horizontal method for the detection and enumeration of Listeria monocytogenes

Chromogenic Coliforms Agar (CCA) ISO

Cat. 2080

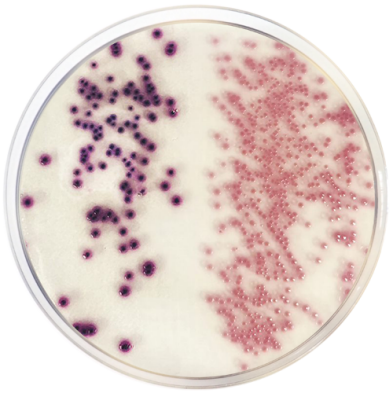
Selective medium for the simultaneous detection of E. coli and other coliforms in water samples.

Practical information

Aplications	Categories
Selective enumeration	Coliforms
Selective enumeration	Escherichia coli

Industry: Water

Regulations: ISO 11133 / ISO 9308



Principles and uses

Chromogenic Coliforms Agar (CCA) is a selective medium for the detection of E. coli and other coliforms in waters and foods. The recovery and enumeration of Escherichia coli and coliforms are important indicators of environmental and food hygiene. CCA is especially recomended for waters with low bacterial numbers, whether it is drinking water, disinfected pool water, or finished water from drinking water treatment plants.

The interaction of ingredients in the medium, such as peptone, sorbitol and pyruvate, grants a quick colony growth, including infectious coliforms and also permits the recovery of sublethal thermally injured coliforms. Tergitol-7 inhibits Gram-positive bacteria and some Gram-negative without affecting the coliform bacteria. Sodium chloride maintains the osmotic balance and phosphate salts act as a buffer system. Bacteriological agar is the solidifying agent.

Detection of ß-glucuronidase is widely used to differentiate Escherichia coli, as the enzyme is present in E. coli but not in other member of coliform group. The chromogenic mixture contains chromogenic substrates: Salmon-GAL and X-glucuronide. Coliform enzymes produced, ß-D-galactosidase and ß-D-glucuronidase, cleave these substrates resulting in the different coloration of bacteria colonies. The ß-D-galactosidase cleaves Salmon-GAL substrate, and gives a salmon-red color to the coliform colonies. The ß-D-glucuronidase, enzyme characteristic of E. coli, cleaves X-glucuronide, giving a blue color to these colonies. E. coli has the two enzymes and cleaves both chromogenic substances giving dark blue to violet colonies. Total coliforms are the sum of E. coli colonies plus salmon-red colonies. The addition of tryptophan to the medium allows the performance of the Indole test for further E. coli confirmation.

Formula in g/L

Enzymatic digest of casein	1	Bacteriological agar	10
IPTG	0,1	Salmon-beta-D-Galactoside	0,2
Sodium chloride	5	Sodium pyruvate	1
Sorbitol	1	Tergitol® 15-S-7 surfactant	0,15
Tryptophan	1	X-beta-G-glucuronide CHX salt	0,1
Yeast extract	2	Sodium dihydrogen phosphate x 2H2O	2,2
Di-sodium hydrogen phosphate	2,7		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 26,45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45-50 ºC, homogenize gently and dispense into Petri dishes.

Instructions for use

For the enumeration of E. coli and coliform bacteria according to ISO 9308:
- Filter sample through a membrane .
- Place the membrane filter over a E. Coli Coliforms Chromogenic Agar plate.
- Invert Petri dish and incubate at 36±2 ºC during 21±3 h.
- Count the ß-D-galactosidase colonies (pink to red in color) as presumptive coliform bacterias that are not E. coli
-To avoid false positive results, caused by oxidase-positive bacteria, for example, Aeromonas spp, confirm bacterial colonies through an oxidase-negative reaction.
- The positive colonies ß-D-galactosidase and ß-D-glucuronidase (dark blue to violet) are counted as E. coli.
- The total coliform bacteria count is the sum of oxidase-negative colonies, ß-D-galactosidase-positive colonies (pink to red) and all colonies which dark blue to violet.
- Some Shigella strains contain the enzyme ß-D-glucuronidase and can grow as light blue colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	6,8±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (36±2 ºC / 21±3 h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA.

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Growth	Colorless colonies
Klebsiella aerogenes ATCC 13048	Good growth >70%	Red to pink colonies
Enterococcus faecalis ATCC 19433	Total inhibition	
Escherichia coli ATCC 25922	Good growth >70%	Dark blue to violet colonies
Escherichia coli ATCC 8739	Good growth >70%	Dark blue to violet colonies

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 9308-1/2014 Water quality — Enumeration of Escherichia coli and coliform bacteria —Part 1: Membrane filtration method for waters with low bacterial background flora.
ISO 7218:2007, Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations Byamukama D., Kansiime F., Mach R.L., Farnleitner A.H. Determination of Escherichia coli. (2) Contamination with Chromocult Coliform Agar Showed a High Level of Discrimination Efficiency for Differing Fecal Pollution Levels in Tropical Waters of Kampala, Uganda. Appl. Environ.
Microbiol. 2000, 66 pp. 864–868 [3] Geissler K ., M anafi M ., A moros I ., A lonso J.L. Quantitative determination of total coliforms and Escherichia coli in marine waters with chromogenic and fluorogenic media. J. Appl. Microbiol. 2000, 88 pp. 280–285 [4] Ossmer R ., Schmidt W., Mende U. Chromocult Coliform Agar — Influence of Membrane Filter Quality on Performance. Poster presentation, 1999. Congreso de la Sociedad Española de Microbiologia, Granada, Spain (http://www.univie.ac.at/chromogenic/OSSMER.PDF) [5] USEPA: 40 CFR Part 141 (sec. 141.21) Federal Register/Vol. 67, No. 209, Tuesday October 29,
2002/Rules and Regulations [6] Lange B ., S trathmann M ., O ssmer R. Performance validation of chromogenic coliform agar for the enumeration of Escherichia coli and coliform bacteria. Lett. Appl. Microbiol. 2 013, 57 pp. 547–553 (http://onlinelibrary.wiley.com/doi/10.1111/lam.12147/suppinfo) [7] http://www.wfcc.info/pdf/WDCM_Reference_Strain_Catalogue.pdf (viewed 03-01-2014)

Condalab

Reference: 5209 **Technical Data Sheet**

Product: **POTASSIUM TELLURITE SOLUTION 1%**

Specification

Aqueous solution of potassium tellurite at 1%, sterilized by filtration and suitable for use as an inhibitor additive in culture media.

Presentation

	Packaging Details	Shelf Life	Storage
1 Bottle 125 ml bottle with: 100 ± 3 ml	1 box with 1 bottle (amber) 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	18 months	15-25 ºC

Composition

Potassium tellurite..... 10.0
Sterile water..... 1000 ml

Reagent to be added, as inhibitor, into culture media for staphylococci isolation.

Description /Technique

Potassium Tellurite Solution is added to culture media as an inhibitor. Its purpose is to prevent the growth of most Gram negative bacteria and of those Gram positive bacteria unable to reduce it.
It is used in media such as Giolitti-Cantoni Broth, Vogel-Johnson Agar, Baird Parker and other selective media for staphylococci. This solution is also contained in selective media for corynebacteria, streptococci and vibrios.
There is a high correlation between the ability to reduce potassium tellurite to tellurium and the pathogenicity of staphylococci, therefore, the presence of potassium tellurite in a medium, together with other tests, helps to determine staphylococci of clinical interest. Potassium Tellurite Solution should be stored at room temperature, since low temperatures will cause crystallization and later precipitation of the product. Should this occur, intense agitation will help redissolve the sediment. Due to its thermolability, the potassium tellurite It is recommended not to subject it to temperatures above 40ºC.

Note: This product is transparent but precipitates may appear without affecting perfomance of the medium

Quality control

Physical/Chemical control

Color : Transparent/colourless pH: at 25ºC

Microbiological control

Add supplement to functionality - into Giolitti Cantoni Broth base

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 37 ºC ± 1, reading after 24-48 ± 2h

Microorganism	Growth
Stph. aureus ATCC® 25923, WDCM 00034	Good - Black precipitate
Staphylococcus aureus ATCC® 6538, WDCM 00032	Good - Black precipitate
Escherichia coli ATCC® 8739, WDCM 00012	Inhibited

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· ATLAS R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
· BAIRD-PARKER, A.C. (1962) An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bact. 25:12.
· COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. USA.
· EUROPEAN PHARMACOPOEIA (2007) 5thed. Suppl. 5.6 § 2.6.13 Microbiological examination of non-sterile products. EDQM. Council of Europe. Strasbourg.
· FIL-IDF 60:2001 Standard. Lait et produits à base de lait - Detection des staphylocoques à coagulase positive - Technique du nombre le plus probable. Brussels.
· ISO 5944:2001 Standard. Milk and Milk based products - Detection of coagulase positive staphylococci - MPN Technique. Geneva.
· ISO 6888-1:1999 Standard. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci - Part 1 Technique using Baird-Parker Agar medium. Geneva.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· ISO 22718 Standard (2015) . Cosmetics - Microbiology - Detection of Staphylococcus aureus.
· USP 31 - NF 26 (2008) <61> Microbial Limit Tests. US Phamacopoeial Conv. Inc. Rockville. MD. USA.
· ZANGERL, P. & H. ASPERGER (2003) Media used in the detection and enumeration of *Staphylococcus aureus*. In Handbook.