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.

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

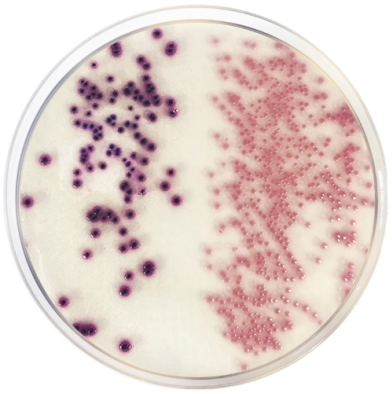
Selective medium for the simultaneous detection of E. coli and other coliforms in water samples.

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.
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Condalab

Reference: 6030 **Technical Data Sheet**

Product: **TTC 1% SUPPLEMENT**

Specification

Redox type indicator of microbial growth.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 6 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-8 ºC

Composition

Compositon (g/vial)

TTC.....0.05

Note: Each vial is sufficient to supplement 2L of Chapman Agar

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....5 ml

Description /Technique

Description:

2,3,5-triphenyltetrazolium chloride (TTC) is a dye largely used for the presumptive detection, isolation and enumeration of microbial colonies like *E. coli* and other coliforms in solid culture media, by the membrane filtration technique in waters for human consumption. Also, the use of TTC is highly recommended for milk testing, because there is a high percentage of microorganisms unable to reduce TTC in pasteurized milk, which cannot be detected by laboratory procedures.
This dye is colorless in the oxidized form and red when reduced by live microorganisms, due to formation of formazan, an insoluble red pigment which is kept inside granules in the cells. Depending on TTC concentration, it may has a very little inhibition of microbial growth in Gram positive organisms.

Technique:

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add 2,5 ml to 1000 ml of TTC Chapman Agar (Cat. 1076), autoclaved and cooled to 45-50 ºC. Mix well and distribute into sterile containers.
When the TTC is required to be added to another media, like KF Streptococcal Agar (Cat. 1034) or KF Streptococcal Agar with Bromocresol Purple (Cat. 1101), refer to the specific instructions of the medium for the quantity of TTC 1% supplement that should be added.

Instructions for use:

For the detection and enumeration of *Escherichia coli* and coliform bacteria in water samples:
- Filter two samples of water over two different membranes and incubate on TTC Chapman Agar (Cat. 1076) at 36±2 ºC and 44±4 ºC respectively for 21±3 hours.

Typical colonies have the appearance as follow:
- *E. coli* and *Citrobacter spp* present yellow colonies with orange-colored center.
- *Enterobacter spp* forms red colored colonies and dark yellow with orange-colored center. The medium is yellow. - *Klebsiella spp* form red colored or yellow, but without center. The medium is yellow.
- Lactose non fermentative bacteria grow with purple colonies and change the medium to blue.

Klebsiella and *Enterobacter* species can also produce yellow-green colonies.
The results will always refer to counts per 100 ml of sample, considering if it has been necessary to make dilutions.
- Count as lactose-positive bacteria the colonies that present a yellow development of the medium under the membrane.
- Subculture the characteristic colonies obtained, in non-selective agar and Tryptophan Culture Broth (Cat. 1237).
- Carry out the oxidase test and incubate the tubes of Tryptophan Culture Broth at 44±0,5 ºC for 21±3 hours.
- Indole production is determined by adding a few drops of Kovac's Reagent (Cat. 5205) to the incubated Tryptophan Culture Broth tubes. A positive test is indicated by the development of red color in the reagent layer.
- The colonies that are oxidase negative will be considered as coliform bacteria and the colonies that are negative oxidase and positive indol will be considered as *E.coli.*

Quality control

Physical/Chemical control

Color : White-yellowish pH: at 25ºC

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely
Add 1 vial to 2L of medium base. DO NOT HEAT once supplemented.
Cool the media at 50 ºC and Pouring into MF dishes.

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

Aerobiosis. Incubation at 36 ± 2 ºC, reading at 21±3 h

Microorganism	Growth
Enterococcus faecalis ATCC® 19433	Inhibited
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥ 70%) Colonies Yellow-orange under MF.
Ps. aeruginosa ATCC® 27853	Good- Red colonies w. blue center.
E. coli NCTC® 13167, WDCM 00179	Good (≥ 70%) Colonies Yellow-orange under MF.

Sterility Control

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

Bibliography

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