

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

Applications	Categories
Selective enumeration	<i>Staphylococcus aureus</i>
Selective enrichment	<i>Staphylococcus aureus</i>

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 strength broth. Suspend 110,4 grams of the medium in one liter of distilled water for the preparation of double strength 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 strength broth and 10 ml for the preparation of double strength broth. Sterilize in an autoclave at 121°C 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 °C 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 °C 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	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Toasted	Amber	6,9 ± 0,2

Microbiological test

According to ISO 11133:

Incubation conditions: Productivity (37±1 °C /24±2 - 48±2 h) / Selectivity (37±1 °C / 48±2 h)

Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10⁴-10⁶ 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 °C
Temp. Max.:25 °C

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.