GIOLITTI-CANTONI BROTH ISO 6888-3, ISO 5944

CAT Nº: 1287 Liquid medium for the enumeration in accordance to the MPN method and selective enrichment of *Staphylococcus aureus* according to ISO 6888-3, ISO 5944

FORMULA IN g/l

D-Mannitol	20.00	Sodium Chloride	5.00
Casein Peptone	10.00	Sodium Pyruvate	3.00
Beef Extract	5.00	Glycine	1.20
Lithium Chloride	5.00	Polysorbate 80	1.00
Yeast Extract	5.00		

Final pH 6.9 ± 0.2 at 25°C

PREPARATION

Suspend 55.2 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. Distribute in 19 ml amounts into and sterilize in autoclave at 121ºC for 15 minutes. Cool to 45-50ºC and aseptically add 0.1 ml of 1% Potassium Tellurite solution per tube. The medium should be stored at 2-8°C. The color is amber.

The dehydrated medium should be homogeneous, free-flowing and toasted in color. If there are any physical changes, discard the medium.

USES

GIOLITTI CANTONI BROTH ISO 6888-3, ISO 5944 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 e ISO 5944 Milk and milk-based products 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.

Incubate the initial suspension for 24 ± 2 h at 37°C. If no blackening develops, incubate for a further 24 ± 2 h. Growth of staphylococci can be recognized by a black coloration of the culture medium due to the reduction of tellurite to tellurium. Subcultivate tubes that present blackening in plates of Baird Parker Agar (Cat. 1319).
Incubate at 37±2ºC and observe after 24-48 hours.

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.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with tellurite added, after incubation at a temperature of 37±2ºC and observed after 24-48 hours.

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Micrococcus luteus ATCC 10240	Inhibited
Staphylococcus aureus ATCC 6538	Good (blackening)
Staphylococcus aureus ATCC 25923	Good (blackening)

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.

ISO

STORAGE

Once opened keep powdered medium closed to avoid hydration.