

Giolitti Cantoni Broth Base

Intended Use:

Recommended for selective enrichment of *Staphylococcus aureus* from suspected food stuffs. The composition and performance criteria are in accordance with ISO 6888-3:2003 and ISO 11133:2014, Amd.2: 2020 (E).

Composition**

ISO Specification Giolitti Cantoni Broth		M584I - Giolitti Cantoni Broth Base	
Ingredients	g / L	Ingredients	g / L
Enzymatic digest of casein	10.000	Tryptone\$	10.000
HM extract #	5.000	HM extract #	5.000
Yeast extract	5.000	Yeast extract	5.000
Mannitol	20.000	Mannitol	20.000
Sodium chloride	5.000	Sodium chloride	5.000
Lithium chloride	5.000	Lithium chloride	5.000
Glycine	1.200	Glycine	1.200
Sodium pyruvate	3.000	Sodium pyruvate	3.000
Polysorbate 80 (Tween 80)	1.000	Polysorbate 80 (Tween 80)	1.000
Final pH (after sterilization)	6.9±0.2	Final pH (after sterilization)	$6.9{\pm}0.2$

**Formula adjusted, standardized to suit performance parameters

\$ Equivalent to Enzymatic digest of casein # Equivalent to Meat extract

Supplements to be added	g / L	Supplements to be added	
Potassium tellurite 0.1 ml of a filter-sterilized	0.050	Potassium tellurite (FD052)	0.1 ml / 9ml
1% aqueous solution of Potassium tellurite per			of tube
9 ml tube			

Directions

Suspend 55.20 gram in 1000 ml purified / distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mmx200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.1 ml of 1% Potassium Tellurite Solution (FD052) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

Principle And Interpretation

Giolitti-Cantoni (1) formulated the broth base and Mossel et al (2) recommended it for detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent in 1 gram of sample. It is also recommended by ISO Committee (3,4) for the examination of meat and meat products.

Mannitol and sodium pyruvate present in the basal medium act as growth stimulants for *Staphylococcus aureus*, aiding in detection of small number of organisms (5). Lithium chloride inhibits gram-negative lactose fermenting bacilli (6). Potassium tellurite and glycine inhibit gram-positive bacilli. Addition of sterile paraffin wax to the inoculated medium inhibits *Micrococci* due to creation of anaerobic conditions. Potassium tellurite concentration must be reduced as per the weight of test sample (0.1 - 0.01 gram).

Inoculate 1 gram of sample or 1 ml of a suitable dilution of a sample into 19 ml of Giolitti-Cantoni Broth tubes in duplicate. Overlay the medium with 5 ml molten sterile paraffin wax and incubate at 37°C for 24-48 hours and examine daily. Blackening of the medium (usually at the bottom) within 48 hours indicates the presence of *Staphylococcus aureus*. The blackened medium, when streaked on Baird Parker Agar (M043I), shows black colonies surrounded by clear zones (7).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

M584I

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The medium should be inoculated as soon as it has been cooled after sterilization, otherwise absorbed oxygen should be expelled by placing the tubes in free-flowing steam for 15 - 20 minutes.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to brownish yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured clear solution without any precipitate.

Reaction

Reaction of 5.52% w/v aqueous solution at 25°C. pH : 6.9±0.2

pН

6.70-7.10

Cultural Response

Productivity : Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) as directed after an incubation at $37\pm1^{\circ}$ C for $24\pm2h$ to 48 ± 2 hours. Recovery is carried out on Baird Parker Agar (M043I) or RPF Agar Base (M1736I).

Selectivity : Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) as directed after an incubation at $37\pm1^{\circ}$ C for 48 ± 2 hours. Recovery is carried out on Tryptone Soya Agar.

Organism	Inoculum (CFU)	Growth	Characteristic reaction on Baird Parker Agar (M043I)
Productivity			
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923	50-100	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
(00034*)+ <i>Escherichia coli</i> ATCC 25922 (00013*)	>=10 ⁴		
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923	50-100	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
(00034*)+ Escherichia coli ATCC 8739 (00012*)	>=10 ⁴		
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)+	50-100	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
<i>Escherichia coli</i> ATCC 25922 25922 (00013*)	>=104		
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)+	50-100	>10 colonies	Black or grey colonies with clear halo (egg yolk clearing reaction
<i>Escherichia coli</i> ATCC 8739 (00012*)	>=10 ⁴		
Selectivity Escherichia coli ATCC 25922	>=10 ⁴	inhibited	
(00013*)			
<i>Escherichia coli</i> ATCC 8739 (00012*)	>=10 ⁴	inhibited	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Giolitti C. and Cantoni C., 1966, J. Appl. Bact., 29:395.

2. Mossel D.A.A., Harrewijn G.A. and Elzebroek J.M., 1973, UNICEF.

3. 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 ISO 6888-3:2003

4.Microbiology of food, animal feeding stuffs and water- Preparation, production, storage and performance culture media, EN ISO 11133:2014 /Amd.2 : 2020 (E).

5.Baird-Parker, A.C., 1962, J.Appl.Bact., 25:12.

6.Lambin S. and German A., 1961, 'Precis de Microbiologie', pg. 63, Paris Masson.

7.De Waart J., Mossel D.A.A., Ten Broeke R. and Van de Moosdijk A., 1968, J.Appl, Bact. 31:276.

8.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

9.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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