GIOLITTI CANTONI STAPHYLOCOCCI BROTH

Dehydrated culture medium

1 - INTENDED USE

Selective enrichment broth for detection and enumeration of coagulase-positive staphylococci in foodstuffs

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.0 g
Beef extract	5.0 g
Yeast extract	5.0 g
Lithium chloride	5.0 g
D-Mannitol	20.0 g
Sodium chloride	5.0 g
Glycine	1.2 g
Sodium pyruvate	3.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococcus aureus has been identified as the causative agent in many foods poisoning outbreaks. Processed foods may contain relatively small numbers of debilitated viable cells, whose presence must be demonstrated by appropriate methods.
Giolitti Cantoni Staphylococci Broth is based on the formulation devised by the Italian microbiologists Giovanni Giolitti and Carlo Cantoni for the recovery of low numbers of stressed *S.aureus* in foods[1,2] and applied by Mossel[3] for the detection of *S.aureus* in dried milk and infant foods.
It is recommended by ISO 6888-3[4] for the detection or enumeration with MPN technique of coagulase positive staphylococci in foodstuffs, where staphylococci are expected to be stressed and in low numbers. Compared to the classic formula of Giolitti and Cantoni, the medium proposed by ISO 6888-3 additionally contains sorbitan mono-oleate.
Essential growth factors are provided by tryptone, beef extract and yeast extract. D-mannitol is the carbohydrate source. Sodium pyruvate and sorbitan mono-oleate aid in resuscitation of stressed cells. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Gram-negative bacteria are inhibited by lithium chloride while Gram positive contaminants are inhibited by the combination of glycine and potassium tellurite added to the medium base. The anaerobic conditions created by paraffin oil layered on the tubes restrict growth of micrococci. Tellurite is reduced by *S.aureus* and related species to tellurium giving the medium a black colour.[5]

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 54.2 g (or 108.4 g in the case of double strength medium) in 1000 mL of cold purified water. If required, add 1 mL (2 mL in the case of double strength medium) of sorbitan mono-oleate (Tween 80 REF 42120502). Warm slightly to completely dissolve the powder. Dispense the single strength medium in quantities of 9 mL into tubes of suitable dimensions (e.g. 16 mm × 160 mm) and 10 mL of double-strength medium in 20 mm x 200 mm tubes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 44 °C to 47 °C and aseptically add to each tube of single strength medium 0.1 mL of a filter sterilised Potassium Tellurite 1% solution (REF42211501) and 0.2 mL to a double-strength medium.
After inoculation, cover the surface of the tubed broth with a 30 mm layer of sterile plain agar or paraffin oil.
If the medium base is stored before the addition of potassium tellurite solution, shortly before use heat it for 15 min at 100 °C to expel air.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Giolitti Cantoni Staphylococci Broth	Dehydrated medium	4015162	500 g (9.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, sorbitan mono-oleate (Tween 80 REF 42120502), Potassium Tellurite 1% Solution (REF 42211501), paraffin oil, Agar Bios LL (REF 411030), ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[4]

9 - TEST PROCEDURE

1. Following the procedure for enumeration and detection by MPN given by ISO 6888-3[4], inoculate 9 mL of single-strength Giolitti Cantoni Staphylococci Broth with 1 g or 1 mL of the initial suspension or 10 mL of double-strenght broth with 10 mL or 10 g sample. For larger volumes of test portions, add the test portion of x g or x mL to 9x g or 9x mL of single-strength broth, previously de-aerated and with potassium tellurite added, and have a minimum air volume in the flask or container.
2. Proceed as specified above for each of the subsequent dilutions.
3. Carefully mix the inoculum and medium, in each case avoiding the introduction of air.
4. Carefully pour a plug of agar, cooled to between 44 °C to 47 ºC, onto the top of the medium in each inoculated tube and allow it to solidify to form a seal. 30 mm layer of sterile paraffin oil may be used instead of agar solution.
5. Incubate the inoculated tubes of at 37 °C ± 1 ºC for 24 h ± 2 h. Subculture any tubes showing any blackening or black precipitate.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

6. Incubate the remainder of the inoculated tubes for a further 24 h ± 2 h and subculture all tubes (i.e. those that do or do not develop a black precipitate after 48 h ± 2 h).
7. For subculturing, aseptically remove the plug of agar or paraffin by using a sterile spatula.
8. With a sterile loop, spread a loop full of each selected broth onto the surface of separate plates of Baird Parker Agar (REF 541116) or Baird Parker RPF Agar (REF 543101) to obtain isolated colonies. Invert the prepared dishes and incubate at 37 °C ± 1 ºC for 24 h ± 2 h and 48 h ± 2 h.

10 - READING AND INTERPRETATION

Formation of a black precipitate or the blackening of the broth suggests the presence of coagulase positive staphylococci.
For the reading and interpretation of plating-out media and for confirmation tests consult the instructions for use of Baird Parker Agar and Baird Parker RPF Agar and follow the procedure given by ISO 6888-3.[4]

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538 P	37°C/48 H/A	growth, blackening of the broth
E. coli ATCC 25922	37°C/48 H/A	no growth after subculture in Tryptic Soy Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Giolitti Cantoni Staphylococci Broth is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 48 hours and recording the highest dilution showing growth and blackening in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *S. aureus* ATCC 25923, *S. aureus* CBS100, *S, aureus* CBS6. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit a good blackening.
Productivity and selectivity are tested together with mixtures of ≤ 100 CFU of target organisms and ≥ 1000 CFU of non- target organisms per test tubes, incubating at 37°C for 48 hours. Mixtures of target and non-target strains: *S. aureus* ATCC 25923+*E. coli* ATCC 25922 and *S. aureus* ATCC 6538+*E. coli* ATCC 25922. After incubation of inoculated tubes and sub-culture on Baird-Parker Agar plates, the target strains will show more than 10 colonies per plate.
Moreover, selectivity is tested by inoculating ≥ 1000 CFU per tube of the following non-target strains: *E. faecalis* ATCC 19433, and *E. coli* ATCC 25922. After incubation the growth of non-target strains is partially inhibited.

13 – LIMITATIONS OF THE METHOD

- A lot of literature has shown that exist false negative and also false positive results of tellurite reduction test (blackening of the medium) and therefore all tubes have to be confirmed by streaking on selective agar media.[4]
- Coagulase enzyme is primarily produced by *S.aureus* but also *Staphylococcus intermedius* and some strains of *Staphylococcus hyicus* are positive to coagulase test.[4]

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (tubes/bottles) and the applied storage conditions (temperature and packaging). According to Curtis GDW *et al*. the prepared medium base may be stored 2 weeks at +4°C, while the complete medium should be used the same day of preparation.[5]

16 – REFERENCES

1. Giolitti G, Cantoni C. Att Soc Sci Vet 1965;19: 509
2. Giolitti G, Cantoni C. A medium for the isolation of staphylococci from foodstuffs. J Appl Bacteriol 1966 Aug; 29(2):395-8
3. Mossel DAA, Harrewijn GA, Elzebroek JM. 1973 UNICEF
4. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase positive staphylococci (Staphylococcus aureus and other species) – part 3: MPN technique for low number.
5. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II) Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:247-8.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it