

Specification

Liquid medium used for the recovery and enumeration of low numbers of coagulase-positive staphylococci in foods according to ISO.

Formula * in g/L

Casein peptone.....	10.0
Meat extract.....	5.0
Yeast extract.....	5.0
Lithium chloride.....	5.0
D-Mannitol.....	20.0
Sodium chloride.....	5.0
Glycine.....	1.2
Sodium pyruvate.....	3.0
Polysorbate 80.....	1.0

Final pH 6,9 ±0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 55.2 g of powder in 1 L of purified water. The medium can be prepared at single strength or double strength by using double the quantity of powder. Distribute into tubes dispensing 10 mL/tube (double strength) or 20 mL/tube (single strength). Sterilise by autoclave at 121°C for 15 minutes. Cool (45-47 °C) and add 1% Potassium Tellurite Sterile Solution (Art. No. DSHB3190) using 0,1 mL/tube.

Description

This medium for the selective enrichment of staphylococci was formulated in 1966 by Giolitti and Cantoni. The growth of staphylococci is promoted by pyruvate, glycine and above all by a high concentration of mannitol. Addition of Polysorbate 80 is necessary for the successful recovery of *Staphylococcus aureus* (Chopin *et al.*, 1985). Accompanying flora are inhibited by lithium chloride and potassium tellurite. Anaerobic growth conditions increase the selectivity of the medium. Generally, growth of staphylococci can be recognized by a blackening or black precipitates in the culture medium due to reduction of tellurite to metallic tellurium. The prepared basal culture medium (without sodium tellurite) can be stored for about 1-2 weeks in the refrigerator. The ready-to-use medium (with the sodium tellurite added) must be used on the same day of preparation. It is advisable that the stored medium base be degasified at the moment of use by heating for 15 minutes at 100°C, cooled rapidly and sterile potassium tellurite solution added.

Technique

Refer to the standard protocol for specific products (Food and animal feeding stuffs EN-ISO 6888-3:2003; Milk and milk based products ISO 5944:2001 and FIL-IDF 60:2001). As a general technique the following is suggested: Use food macerates or a 10-fold serial dilution and inoculate 1 mL in single strength medium. To lower the detection limit, 10 mL of the test sample (liquid products) or the first dilution (other products) may be inoculated in double strength medium. MPN procedures need at least three tubes for at least three dilution steps. If no anaerobic jar is available, overlay with a layer of sterilized vaseline or vaspar. Incubate anaerobically for 24-48 hours at 37°C. After 24 hours, subculture any tubes showing blackening or black precipitate by streaking onto Baird-Parker Agar. Incubate the remainder of the tubes for a further 24 hours and subculture all tubes showing growth (irrespective of blackening) to Baird-Parker Agar. When determining the bacterial count by the MPN method, all tubes showing growth are considered as presumptive positive for staphylococci and they are confirmed only if they produce a positive result in the coagulase test.

Quality control

Incubation temperature: 37°C ±1,0

Incubation time: 24 - 48 ± 2h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism

Staphylococcus aureus ATCC® 25923
Staphylococcus aureus ATCC® 6538
Escherichia coli ATCC® 8739
Staph. aureus (25923) + *E. coli* (8739)

Growth

Good
 Good
 Inhibited
 Good

Remarks

Black precipitate to 48 h in BP Agar
 Black precipitate to 48 h in BP Agar
 Recovery in TSA
 > 10 CFU *Staph. aureus* ; recovery in BP Agar

References

- CHOPIN, A. et al. (1985) ICMSF Methods Studies XV. Comparison of four media and methods for enumerating *Staphylococcus aureus* in powdered milk. J. Food Protect. 48:21-27.
- EN-ISO 6888-3 Standard (2003) 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.
- FIL-IDF (2001) Milk and milk based Products. Detection of coagulase-positive staphylococci. MPN technique. Standard 60:2001. Brussels.
- GIOLITTI, G. A. CANTONI, C. (1966) A medium for the isolation of staphylococci from foodstuffs. J.Appl. Bact. 29, 395-398.
- HARRIGAN, WF. & McCANCE, M.E. (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press. London.
- ISO 5944 Standard (2001) Milk and milk based Products. Detection of coagulase-positive staphylococci. MPN technique.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).