TECHNICAL DATA SHEET

GIOLITTI & CANTONI BROTH WITH TWEEN 80 (BASE)

ENRICHMENT AND ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI

1 INTENDED USE

Giolitti and Cantoni Broth with Tween 80 is a selective enrichment medium for the detection and enumeration (particularly in low numbers) of coagulase positive staphylococci in food products. The media is used with the MPN method.

The typical composition corresponds to that defined in the standard NF EN ISO 6888-3.

2 HISTORY

Mossel, Harrewijn and Elzebrock recommended the medium formulated by Giolitti and Cantoni in 1966 for the detection of *Staphylococcus aureus* in powdered milk and baby formulas. The present formula, supplemented with Tween 80, has been recommended by Chopin *et al*. (in 1985) to increase the media productivity.

3 PRINCIPLES

Pyruvate, glycine and especially mannitol favor the development of staphylococci.

Gram-negative bacteria are inhibited by lithium chloride.

Gram-positive strains are inhibited by the action of potassium tellurite.

The anaerobic environment suppresses the growth of micrococci.

Tween 80 acts as a dispersion agent.

The development of staphylococci is shown by the appearance of a black color due to the reduction of tellurite to metallic tellurium.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of base media (without potassium tellurite) :

- Tryptone ..... 10,0 g
- Meat extract..... 5,0 g
- Yeast extract ..... 5,0 g
- Glycine ..... 1,2 g
- Mannitol..... 20,0 g
- Sodium pyruvate ..... 3,0 g
- Sodium chloride ..... 5,0 g
- Lithium chloride ..... 5,0 g
- Tween 80..... 1,0 g
- Potassium tellurite (*****) ..... 0,1 g

pH of the ready-to-use media at 25 °C : 6,9 ± 0,2.

(*****) : Potassium tellurite is not included in the base media composition and must be added just before inoculation.

5 PREPARATION

Preparation of the potassium tellurite solution

- Suspend 1 g of potassium tellurite in 100 mL of distilled or demineralized water.
- Shake until complete dissolution.
- Filter through a 0,22 µm membrane and recover the solution in a sterile vial.

Preparation of the media in single strength concentration :

- Dissolve 55,2 g of dehydrated media (BK159) in 1 liter of distilled or demineralized water.
- Slowly bring to a boil, with constant agitation until complete dissolution.
- Dispense into tubes at 10 mL per 16 x 160 mm tube.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution : 55,2 g/L**

✓ **Sterilization : 15 min at 121 °C**

Preparation of the media in double strength concentration :

- For double strength concentration, dissolve 110.4 g of dehydrated medium (BK159) in 1 liter of distilled or demineralized water.
- Slowly bring to a boil, with constant agitation until complete dissolution.
- Dispense into tubes at 10 mL per 20 x 200 mm tube.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution : 110,4 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of ready-to-use media

- When the media has been prepared in advance or when ready to use media is used (BM110 and BM111), de-gas the basic medium (single or double strength) before use by heating for 15 minutes at 95-100 °C, just before use.
- Cool and maintain at room temperature.

Preparation of agar overlay

- Dissolve 15 to 20 g of agar (A1010 or A1012) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into tubes or vials.
- Autoclave 15 min. at 121 °C
- Cool and maintain in a molten state at 44-47 °C

6 INSTRUCTIONS FOR USE

Double strength medium :

- Add to each tube 0.2 mL of the sterile 1% potassium tellurite solution.
- Transfer 10 mL of inoculum to each tube of double strength medium.

Single strength medium:

- Add to each tube 0.1 mL of the sterile 1% potassium tellurite solution.
- Transfer 1 mL of inoculum and its serial dilutions to each tube of single strength medium.

✓ **Inoculation :**
- Add tellurite
- Single strength : 1 mL
- Double strength : 10 mL

✓ **Incubation : 24 h and 48 h at 37 °C**

- Mix well, avoiding trapping air bubbles.
- Pour a layer of sterile molten (44-47°C) agar into the tubes.
- Let cool and incubate 24 h at 37 °C.
- If the tubes present no blackening or precipitate at 24 hours, prolong the incubation another 24 hours.

7 RESULTS

Inoculate tubes demonstrating blackening or a black precipitate after 24 h of incubation onto Baird-Parker Agar with Egg Yolk Tellurite (BM018) or onto prepared Baird Parker RPF plates (BM067).
After 48 hours of incubation, re-inoculate all the other tubes.
All plates should be incubated for 48 hours at 37 °C.
The presence of characteristic colonies confirms the presence of coagulase positive staphylococci in the tubes. Calculate the Most Probable Number or the presence of the specific microorganism.

8 QUALITY CONTROL

Dehydrated media : light beige powder, free-flowing and homogeneous.
Prepared media : amber solution, limpid.

Typical culture response after 48 hours of incubation in anaerobic conditions at 37 °C, followed by subculture according to NF EN ISO 11133 :

Microorganisms		Growth
Staphylococcus aureus + *Escherichia coli*	WDCM 00034 WDCM 00013	> 10 characteristic colonies
Escherichia coli	WDCM 00012	Inhibited

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Ready-to-use base media (single & double strength) in tubes : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base media in tubes (*): 180 days at 2-8 °C.
Complete prepared media in tubes with tellurite (*) : use on the day of preparation.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without potassium tellurite) :
500 g bottle BK159HA

Ready-to-use base media (without potassium tellurite) :
50 x 10 mL tubes (single strength media)..... BM11008
50 x 10 mL tubes (double strength media) BM11108

American type Agar :
500 g bottle A1010HA
European type Agar :
500 g bottle A1012HA

11 BIBLIOGRAPHY

Giolitti, G., and Cantoni, C.. 1966. A medium for the isolation of staphylococci from foodstuffs. Journal of Applied Bacteriology, **29** : 395-398.

NF EN ISO 6888-3. Juin 2003. Microbiologie des aliments. Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces). Partie 3 : Recherche et méthode NPP pour les faibles nombres.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : GIOLITTI & CANTONI_EN v9.
Creation date : 01-2004
Updated : 05-2016
Origin of revision : General update.