



Technical Data

Broth Medium I (Tetrathionate Bile Brilliant Green Broth)

M1255B

Tetrathionate Bile Brilliant Green Broth (Broth Medium I) is used for isolation and identification of *Salmonellae* in accordance with British Pharmacopoeia, 2009.

Composition**

Ingredients	Gms / Litre
Peptone	8.600
Ox bile dried	8.000
Sodium chloride	6.400
Calcium carbonate	20.000
Potassium tetrathionate	20.000
Brilliant green	0.070
pH after heating (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 63.07 grams in 1000 ml purified/ distilled water. Heat just to boiling. DO NOT AUTOCLAVE OR REHEAT. Dispense as desired.

Note: Due to presence of Calcium Carbonate, the prepared medium forms opalescent solution with white precipitate .

Principle And Interpretation

Tetrathionate Bile Brilliant Green Broth cited as Broth Medium I is prepared as per the recommendation of British Pharmacopoeia. (1) Is used for isolation and identification of *Salmonella* species in the tests prescribed for sterility checking in the Pharmacopoeia. It is also used to detect *Salmonella* from pharmaceutical, foods, water and other materials of sanitary importance.

Peptone provides nitrogenous nutrients to the *Salmonellae* . Brilliant green and ox-bile inhibit both gram-positive as well as some selected gram-negative organisms. Potassium tetrathionate inhibits normal flora of faecal specimens. Sodium chloride helps in maintaining osmotic equilibrium. Calcium carbonate neutralizes the acids produced by reduction of tetrathionate.

Medium is not suitable for growth of *Salmonella* Typhi and *Salmonella* Paratyphi (2). The sample is initially enriched in Broth Medium I and incubated at 43-45°C for 18-24 hours. The enriched sample is then subcultured on any two of following Agar medium J , Agar medium K and Agar medium L.

Quality Control

Appearance

Light yellow to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Bluish green coloured opalescent solution with white precipitate.

pH

6.80-7.20

Cultural Response

M1255B: Cultural characteristics observed after enrichment in Broth Medium I at 41-43°C for 18-24 hours, and then subcultured on Agar Medium K (M031B) and Agar Medium L (M016B) and incubated at 35-37°C for specified period.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
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Cultural Response

Growth on Agar Medium**K**

<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	>=10 ³	inhibited	0	0%		>=72 hrs
<i>Escherichia coli</i> ATCC 8739	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

Growth on Agar Medium**L**

<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	25 -100	>=50 %	pinkish white	18 -72 hrs
<i>Salmonella Abony</i> NCTC 6017	50 -100	luxuriant	25 -100	>=50 %	pinkish white	18 -72 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	>=50 %	pinkish white	18 -72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	>=10 ³	inhibited	0	0%		>=72 hrs
<i>Escherichia coli</i> ATCC 8739	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. British Pharmacopoeia, 2009, The Stationery office British Pharmacopoeia
2. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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