



Broth Medium I (Tetrathionate Bile Brilliant Green Broth)

ME1255

Intended Use:

Recommended for isolation and identification of *Salmonella* in accordance with EP.

Composition**

Ingredients	g / L
Peptone	8.600
Bile, dried #	8.000
Sodium chloride	6.400
Calcium carbonate	20.000
Potassium tetrathionate	20.000
Brilliant green	0.070
pH after heating	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Ox-bile dried

Directions

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

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

Principle And Interpretation

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

Peptone provides nitrogenous nutrients to the *Salmonellae*. Brilliant green and Bile, dried 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). After incubation streak onto two of any of the selective like Agar Medium J, Agar Medium K, or Agar Medium L. Incubate at 35-37°C for 18-72 hours and observe for typical colony appearance and characteristics (1).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

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. For further confirmation, streak the enriched cultures after incubation, on plates of Brilliant Green Agar (M016), MacConkey Agar (M081) and Bismuth Sulphite Agar (M027).

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.

Please refer disclaimer Overleaf.

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Bluish green opalescent solution with white precipitate

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after enrichment in Broth Medium I at 41-43°C for 18-24 hours, and then subcultured on Agar Medium K (Xylose Lysine Deoxycholate Agar),(ME031) and Agar Medium L (Brilliant Green, Phenol red, lactose monohydrate Sucrose Agar), (ME016) and incubated at 35-37°C for 18-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	Incubation period
Growth on Agar Medium K					
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	>=50 %	red with black centres	18 -72 hrs

Additional Microbiological Testing Growth on Agar Medium K

<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	red with black centres	18 -72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ³	inhibited	0%		>=72 hrs
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	fair	20 -30 %	yellow	18 -72 hrs

Growth on Agar Medium L

<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	pinkish white	18 -24 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	luxuriant	>=50 %	pinkish white	18 -24 hrs

Additional Microbiological Testing Growth on Agar Medium L

<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	pinkish white	18 -24 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ³	inhibited	0%		>=24 hrs
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	fair	20 -30 %	yellow	18 -24 hrs

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (3,4).

Reference

1. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.

Revision : 03/2025

Disclaimer :

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