TETRATHIONATE BROTH BASE

Dehydrated culture medium

1 - INTENDED USE

Selective liquid medium for the enrichment of *Salmonella* from food animal feeding stuffs and other samples of sanitary importance.

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

Peptocomplex	5 g
Bile salts	1 g
Calcium carbonate	10 g
Sodium thiosulphate	30 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

Mueller[1] first described the use of Tetrathionate Broth for the isolation of salmonellae. The modification of the original formula, developed by Kauffmann[2], with the addition of bile salts and brilliant green to suppress bacteria such as *Proteus* spp., notably increased the percentage of positive results in the isolation of *Salmonella* spp.
The detection of *Salmonella* in foods and other samples of sanitary interest. necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.
Tetrathionate Broth Base with the addition of iodine-iodide and brilliant green solutions is used for the selective enrichment of *Salmonella* from food animal feeding stuffs and other samples of sanitary importance and conforms to FDA-BAM formulation.[3]
Peptocomplex provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are ox bile, brilliant green and sodium tetrathionate which is formed from sodium thiosulfate when the iodine-potassium iodide solution is added to the medium; calcium carbonate neutralizes the sulfuric acid that is produced by the reduction of tetrathionate during the growth of salmonellae, keeping the pH at neutral values. The complete medium allows the development of salmonellae and is inhibitory for Gram-positive bacteria and for a large part of Gram-negative bacteria of enteric origin.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 46 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Avoid overheating, do not autoclave. Cool to 42-45° and aseptically add 20 mL of iodine-iodide solution and 10 mL of brilliant green 0.1% Solution (REF 421505). Mix well and aseptically distribute 10 mL into sterile tubes. Preparation of iodine-iodide solution according to FDA BAM[4]: dissolve 5 g of potassium iodide in 5 mL sterile purified water; add 6 g of iodine and stir to dissolve; dilute to 20 mL with sterile purified water.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder.
Solution and prepared tubes appearance	colourless to light yellow supernatant over a heavy white precipitate
Final pH of the complete medium at 20-25°C	8.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tetrathionate Broth Base	Dehydrated culture medium	4021252	500 g (10.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile tubes, iodine-iodide solution, Brilliant Green 0.1% Solution (REF 421505), ancillary culture media and reagents.

8 - SPECIMENS

Food, feed, food chain sample and other samples of sanitary. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[3]

9 - TEST PROCEDURE

The following method is a summary taken from the FDA-BAM.[3] In the microbiological examination of foods, FDA-BAM recommends the use of Tetrathionate (TT Broth) together with Rappaport Vassiliadis (RV) Broth or Selenite Cystine Broth (SCB) (for guar gum).
TT Broth is used as the second selective enrichment broth, incubated at 43°C for the analysis of high microbial load foods and at 35°C for the analysis of low microbial load foods.

1. Transfer 0.1 mL of non-selective pre-enrichment culture to 10 mL of RV Broth or to 10 mL of SCB and another 1 mL aliquot to 10 mL of TT Broth.
2. Mix well and incubate selective enrichment media as follows:
   - Foods with a high microbial load: incubate RV Broth 24 ± 2 h at 42 ± 0.2°C. Incubate TT Broth 24 ± 2 h at 43 ± 0.2°C
   - Foods with a low microbial load (except guar gum and foods suspected to be contaminated with S. Typhi): incubate RV Broth 24 ± 2 h at 42 ± 0.2°C. Incubate TT Broth 24 ± 2 h at 35 ± 2.0°C.
   - Guar gum and foods suspected to be contaminated with S.Typhi: incubate SC and TT broths 24 ± 2 h at 35°C.
3. Mix the selective enrichment broths and streak 10 µl incubated TT broth on Bismuth Sulfite Agar, XLD Agar and Hektoen Enteric Agar.
4. Repeat with 3 mm loopful (10 µl) of RV Broth (for samples of high and low microbial load foods) and of SC broth (for guar gum).
5. Incubate plates 24 ± 2 h at 35°C. Examine plates for presence of colonies that may be *Salmonella*.

10 - READING AND INTERPRETATION

Refer to the instructions for use of the plated media for the description of *Salmonella* colony characteristics.
Mark suspect colonies on each plate. Select suspect colonies for subculture and confirmation. For the choice of biochemical tests for the identification of *Salmonella* and the criteria for the evaluation of positive and negative reactions, refer to Chapter 5 of the FDA-BAM.[3]

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Biochemical confirmation may be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

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 of medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028	35-37°C / 21-24h A	good growth after subculture on TSA
E. coli ATCC 25922	35-37°C / 21-24h A	partially inhibited after subculture on TSA

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 Tetrathionate Broth Base, supplemented with iodine and brilliant green solutions, are 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 24 hours, sub-culturing on Tryptic Soy Agar plates and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: S.Typhimurium ATCC 14208, S.Enteritidis ATCC 13076, *S.diarizonae* ATCC 12325. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity and selectivity are tested together with mixtures of approximately 100 CFU of target organisms and 1000 CFU of non- target organisms per test tubes, incubating 37°C for 24 hours. Mixtures of target and non-target strains: S.Typhimurium ATCC 14028 +*E.coli* ATCC 25922+*P. aeruginosa* ATCC 27853. After incubation and sub-culture on XLD Agar plates, the target strains will show more than 10 colonies per plate.Moreover, selectivity is evaluated by inoculating approximately 10,000 CFU/tube of non-target organisms and incubating at 37C for 24 hours and sub-culturing on Tryptic Soy Agar plates. Selectivity is tested with the following non-target strains: *E. coli* ATCC 25922, *E. faecalis* ATCC 2921. CFUs of *E. coli* shall be less than 100 while CFUs of *E. faecalis* shall be less than 10 on the sub-cultured plates of Tryptic Soy Agar.

13 - LIMITATIONS OF THE METHOD

After the enrichment in Tetrathionate Broth, the isolated colonies on the plates should be identified with suitable tests.

14 - PRECAUTIONS AND WARNINGS

- This product 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 culture medium 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 product 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 FDA-BAM iodine and brilliant green solutions should be added to medium base on day of use.[4] According to MacFaddin, Tetrathionate Broth Base without the addition of iodine solution may be stored at +2°C /+8°C in tightly closed containers for up to 2 weeks.[5]

16 – REFERENCES

1. Muller L. A nouveau milieu d'enrichissement pour la recherche du bacille typhique e des paratyphiques. C.R. Soc. Biol. (Paris) 1923; 89:434-443
2. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z Hyg Infektionskr. 1935; 117: 26-32
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 03/2022.
4. U.S. Food and Drug Administration. BAM Media M145: Tetrathionate (TT) Broth.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	
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	2023/02

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

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