INSTRUCTIONS FOR USE

MUELLER KAUFFMANN TETRATHIONATE BROTH

Ready-to-use tubes

Muller KauffmannTetrathionate Broth – from the left: uninoculated tube and tube with growth of S.Typhimurium.

1 - INTENDED USE

In vitro diagnostic device. Selective liquid medium for the enrichment of *Salmonella* from food and faecal specimens.

2 - COMPOSITION -TYPICAL FORMULA *

Tryptone	7.00 g
Soy Peptone	2.30 g
Sodium Chloride	2.30 g
Calcium Carbonate	25.00 g
Sodium Thiosulphate	40.70 g
Bile Salts	4.75 g
Iodine	3.8 g
Potassium Iodide	4.75 g
Brilliant Green	9.5 mg
Purified Water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Muller Kauffmann Tetrathionate Broth has been originally described by Muller[1] and later modified by Kauffmann[2] by the inclusion of ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp.
The medium is included in the reviews ISTISAN 05/27[3] and ISTISAN 96/35[4] for the selective enrichment of *Salmonella* from samples of the food chain and from faeces prior to selective isolation.
Tryptone and soy peptone provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are bile salts, the added brilliant green and sodium tetrathionate which is formed from the 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 - PHYSICAL CHARACTERISTICS

Medium appearance	pale green with white precipitate
Final pH at 20-25°C	8.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Muller Kauffmann Tetrathionate Broth	Ready-to-use tubes	551743	20 x 10 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, needles and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Muller Kauffmann Tetrathionate Broth may be used for the enrichment of faecal specimens. Collect stool according to standard procedures, with swab preferably with transport medium or in a stool container with or without transport fluid. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied
Food samples: refer to applicable Standards and laws.

8 - TEST PROCEDURE

Allow tubes to come to room temperature. For faeces examination, inoculate test tubes with 1 g of faeces, or 1 mL of faecal suspension obtained suspending 1 g of faeces in 1 mL of saline solution. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
Incubate the inoculated tubes in aerobic atmosphere at 35-37°C for 18-24 hours.
For milk and dairy products, the following procedure can be used:
Transfer 25 g of sample to 225 mL of Buffered Peptone Water and incubate at 35-37°C for 18-24 hours.
From the pre-enrichment broth transfer 2 aliquots of 10 mL respectively into 100 mL of Muller Kauffmann Tetrathionate Broth and into 100 mL of Selenite Cystine Broth.
Incubate Muller Kauffmann Tetrathionate Broth at 42-43°C for 24 and 48 hours and Selenite Cystine Broth at 35-37°C for 24 and 48 hours. After 24 and 48 hours of incubation subculture on appropriate selective enteric media.

9 - READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity and discolouration. Subculture by streaking a loopful of broth on selective enteric plating media. The plating media should be chosen as a combination of greater and lesser inhibitory selective agars.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it

10 - 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 / 18-24h / A	good growth after subculture to TSA plate
E.coli ATCC 25922	35-37°C / 18-24h / A	scanty growth after subculture to TSA plate

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use tubes of Muller Kauffmann Tetrathionate Broth and of the raw material used for the production of prepared tubes (dehydrated Muller Kauffmann Tetrathionate Broth Base REF 401743 supplemented with brilliant green and iodine 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 and incubating at 35-37°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}) after sub-culture on Tryptic Soy Agar plates. Productivity is tested with the following target strains: S.Typhimurium ATCC 14028, S.Enteritidis ATCC 13076. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

Selectivity is evaluated with dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in duplicate test tubes and incubating at 35-37°C and at 42-43°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}) after sub-culture on Tryptic Soy Agar plates. Selectivity is tested with the following non-target strains: *E.coli* ATCC 25922, *E.faecalis* ATCC 29212. The selectivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≥1.

Productivity and selectivity are tested also together with a mixture of appropriate dilutions of target and non-target strains: S.Typhimurium ATCC 13076+*E.coli* ATCC 25922+*P.aeruginosa* ATCC 27853. After incubation of inoculated tubes at 35-37°C and at 42-43°C for 18-24 hours and subculture on XLD Agar plate, the target strain will show a predominant growth on plated medium.

12 - LIMITATIONS OF THE METHOD

- Muller Kauffmann Tetrathionate Broth is not suitable for growth of S.Typhi, S.Paratyphi, S.Sendai, S.Gallinarum; it is not recommended for examination of typhoid fever.[5]
- After the enrichment in Muller Kauffmann Tetrathionate Broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Muller Kauffmann Tetrathionate Broth Base is sterilized by autoclaving and the tubes are added with the supplements under aseptic conditions; the tubes cannot to be considered a "sterile product", but a product with controlled bio-contamination, within the limits of the defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour).

15 – REFERENCES

1. Muller, L. (1923) C.R. Soc. Biol. (Paris) **89**, 434-443
2. Kauffmann, F. (1935) Z.f. Hyg. **117**, 26-32
3. Rapporto ISTISAN 05/27. ISSN 1127-3117. Infezioni da Salmonella: diagnostica, epidemiologia e sorveglianza. Raccolta a cura di C.Graziani, P.Galetta, L.Busani, AM Dionisi, E.Filetici, A.Ricci, A.Caprioli, I.Luzzi.
4. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D. De Medici, L. Fenicia, L. Orefice e A.Stacchini.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/05
Revision 2	Removal of obsolete classification	2023/04

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