

Specification

Medium used for the selective enrichment of salmonellae, according to ISO standard 6579.

Presentation

	Packaging Details	Shelf Life	Storage
20 Tubes Tube 16 x 113 mm with: 10 ± 0.3 ml	16x113 mm glass tubes, ink labelled, metal cap. - 20 tubes per box	12 months	8-25 °C

Composition

Composition (g/l):	
Bile salts No. 3.....	4.7800
Meat extract.....	4.3000
Casein peptone.....	8.6000
Sodium chloride.....	2.6000
Calcium carbonate.....	38.700
Sodium Thiosulfate.5 H ₂ O.....	47.800
Brilliant Green.....	0.0096
Novobiocin.....	0.0400

Description /Technique

Tetrathionate Broth is a classic medium for the enrichment of enteric or intestinal pathogens, including all members of *Salmonella spp.*, from heavily polluted samples, such as faeces, urine, waste water and others. During preparation, when iodine is added, tetrathionate is produced from the sulfate, and this salt together with the bile salts in the medium, results in a strong inhibition of most of the normal intestinal bacteria, except for those which are capable of reducing tetrathionate, e.g. salmonellae. Reduction reactions liberate sulphuric acid, which is neutralized by the carbonate, avoiding a decrease in the pH, which is harmful even for salmonellae.

However, many *Proteus* species resist the bile salt concentration and, they may reduce tetrathionate. So, many authors recommend the addition of other inhibitors simultaneously, such as 0,1% Brilliant Green Solution (10 mL/L) and/or novobiocin at 40 mg/L.

Technique:

Note: White precipitate is due to calcium carbonate and does not effect the broths performance.

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Immediately before inoculation of Müller Kaufman Tetrathionate Novobiocin broth formulated according to ISO 6579:2017, supplement tubes/bottles to get the following concentrations of Iodine/Iodide solt.:

2 ml of Iodine-Iodide solution to each 100 ml medium base in bottles

0.2 ml of Iodine-Iodide to each 10 ml medium base in tubes.

Inoculate aseptically the tubes / bottles with the prepared sample or its dilution and incubate the tubes tightly closed aerobically at 37±1 °C for 24±3h. Discard supplemented tubes if not used in less than 10 hours.

After enrichment incubation, noculate any confirmatory, secondary medium by streaking methodology or by spiral method, like, XLD. Enumerate all the colonies that have appeared onto the surface of the agar. Presumptive isolation / recovery of Salmonella must be confirmed by further microbiological and biochemical tests.

Each laboratory must be evaluate the results according to their specifications.

Quality control**Physical/Chemical control**

Color : Greenish - White pH: 8.0 ± 0.2 at 25°C

Microbiological control

Add supplement to functionality - Inoculate : Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/10⁴-10⁶ (selectivity).

Add 20ml/l of yodo iodine solution

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 36 ± 2 °C, reading at 24 ± 3 h

Subculture after incubation onto appropriate media

Microbiological control according to ISO 11133:2014/A1:2018.

Microorganism

Enterococcus faecalis ATCC® 29212, WDCM 00087

Escherichia coli ATCC® 8739, WDCM 00012

S. typhimurium (14028) + *E. coli* (8739) + *Ps.* (27853)

S. enteritidis (13076) + *E. coli* (8739) + *Ps.* (27853)

Sterility control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Growth

Inhibited- < 10 CFU Recovery in TSA;

Partially Inhibited ; ≤ 100 CFU Recovery in TSA

Salmonella coln. charact. in XLD (37°C±1 / 24 ± 3h) ≥ 10

Salmonella coln. charact. in XLD (37°C±1 / 24 ± 3h) ≥ 10

Bibliography

- DIN Standard 10160 Untersuchung von Fleisch und Fleischerzeugnissen: Nachweis von Salmonellen. Referenzverfahren.
- DIN Standard 10181 Mikrobiologische Milchuntersuchung: Nachweis von Salmonellen. Referenzverfahren.
- DOWNES, F.P. & K.I.TO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. DC. USA.
- FDA (Food and Drug Administration) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- FIL-IDF Standard 93 (2001) Milk and milk products: Research of *Salmonella*.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburg. MD. USA.
- ISENBERG, H.D. (1992) Clinical Microbiology Procedures Handbook. Vol. 1. APHA. Washington. DC. USA.
- ISO Standard 6579-1 (2017) Microbiology of food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 : Detection of *Salmonella* spp.
- ISO Standard 6785 (2001) Milk and Milk Products - Detection of *Salmonella* spp.
- ISO Standard 3565 (1975) Meat Products: Reference Method for detection of Salmonellae.
- ISO 11133:2014/ Adm 1:2018./ Adm1:2018 Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- KAUFFMAN, F. (1931) Ein Kombiniertes Anreicherungsverfahren für Typhus und Paratyphus Bazillen. Zblt. Bakt Microbiol. Hyg Abt. I. Orig. 119:148.
- MARSHALL, R.T. (1993) Standard methods for the examination of dairy products. 16th ed. APHA Washington. DC. USA.
- MULLER, L. (1923) Un nouveau milieu d'enrichissement pour la recherche du bacille typhique est des paratyphiques. Comp. Rend. Soc. Biol. 89:434-437.
- U.S. PHARMACOPOEIA (2002) 25th ed. <61> Microbial Limits Test. US Pharmacopeial Convention Inc. Rockville. MD. USA.