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Muller-Kaufmann Tetrathionate-Novobiocin (MKTTn) Broth
700004511, 700004512, 700004513, 700004514
(NCM0126)

Intended Use
Muller-Kauffmann Tetrathionate-Novobiocin Broth is used for the selective and differential isolation of *Salmonella* spp. in a laboratory setting and is included in ISO 6579:2017. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

Description
A selective enrichment medium for the detection of *Salmonella* spp. in food, animal feed and in environmental samples from the food production area as described in ISO 6579-1:2017. Mueller originally described a tetrathionate broth that inhibited lactose fermenting Enterobacteriaceae but not *Salmonella*. Kauffmann later modified the formulation to include ox bile and brilliant green to improve selectivity. Finally, Jeffries added Novobiocin to inhibit *Proteus* species.

Meat extract and casein provide a source of nitrogen and amino acids and sodium chloride maintain the osmotic balance. Ox bile and brilliant green act as selective agents against non-target microorganisms. Tetrathionate is generated from the sodium thiosulfate. Iodine and calcium carbonate buffer the sulfuric acid generated from tetrathionate reduction. According to ISO 6579-1:2017 subculture is performed from Buffered Peptone Water (BPW) into MKTTn, followed by subculture onto Xylose-Lysine Deoxycholate (XLD) Agar ISO and second agar. This medium conforms to the performance and formulation requirements of ISO 6579-1:2017.

Typical Formulation

Meat Extract	4.3 g/L
Enzymatic Digest of Casein	8.6 g/L
Sodium Chloride	2.6 g/L
Calcium Carbonate	38.7 g/L
Sodium Thiosulfate (anhydrous)*	30.45 g/L
Ox Bile	4.78 g/L
Brilliant Green	0.0096 g/L

pH: 8.0 ± 0.2 (base medium)
*Equivalent to 47.8g of sodium thiosulfate pentahydrate.
Formula is adjusted and/or supplemented as required to meet performance specifications

Supplement:
NCM4040 or 700004897 Novobiocin

Precaution
Refer to SDS

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Preparation for DCM

1. Dissolve 89.4 grams of the medium in one liter of purified water.
2. Mix thoroughly and bring to a boil.
3. Cool to less than 45°C before adding supplements.
4. Supplement NCM0126 with 20 mL of iodine-iodide solution (see below) and 4 vials of NCM4040-0.5* or 700004897 Novobiocin. NCM3503 is provided fully supplemented so no further additions are required.
5. Mix well and distribute into sterile containers.

Iodine-iodide solution

Dissolve 25g of potassium iodide in 10 ml of water. Add 20g iodine and dilute to 100ml with sterile deionized water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions

Test Procedure

Refer to ISO 6579-1:2017/Amd 1:2020

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, slightly lumpy, and off-white.

Prepared Appearance: Prepared medium is pale green chalky, white precipitate upon standing and cloudy.

Expected Cultural Response: Muller-Kauffmann Tetrathionate-Novobiocin Broth was inoculated with organisms listed below and incubated aerobically at 37 ± 1C for 21 - 27 hours. After incubation in Muller-Kauffmann Tetrathionate-Novobiocin Broth, organisms were sub-cultured to Xylose Lysine Deoxycholate (XLD) Agar ISO (NCM0021), incubated at 37 ± 1C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results Growth
Salmonella typhimurium ATCC® 14028	10 – 100	≥ 10 cfu on XLD
Salmonella enteritidis ATCC® 13076	10 - 100	≥ 10 cfu on XLD
Enterococcus faecalis ATCC® 29212	> 10^4	<10 cfu on TSA
Enterococcus faecalis ATCC® 19433	> 10^4	<10 cfu on TSA
Escherichia coli ATCC® 25922	> 10^4	≤ 100 cfu on TSA
Escherichia coli ATCC® 8739	> 10^4	≤ 100 cfu on TSA
Pseudomonas aeruginosa ATCC® 27853	> 10^4	≥ 10 cfu on XLD

The organisms listed are the minimum that should be used for quality control testing.

Results

Salmonella spp. will produce red colonies with black centers on XLD Agar ISO. On Brilliant Green Agar, Salmonella colonies are typically opaque and pink, and on Chromogenic Agar for Salmonella Esterase (CASE) (NCM1006) Salmonella colonies are blue/green.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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Limitations of the Procedures

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium. Organisms other than *Salmonella* spp., such as members of the *Enterobacteriaceae* may also grow.
2. Muller-Kauffmann Tetrathionate-novobiocin Broth prepared from NCM0126 is unstable and should be used immediately. It may be stored at 2 - 8°C in the dark for no longer than 7 days. NCM3503 ready to use Muller-Kauffmann Tetrathionate-novobiocin Broth is stable up to the shelf life marked on the container if stored at 2 - 8°C in the dark.
3. Confirmatory tests, such as biochemical reactions and serological confirmation are required.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 6579-1:2017/Amd 1:2020 Microbiology of food and animal feeding stuffs – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – *Part 1*

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