MKTTN according to ISO 6579

B.3 Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn)

B.3.1 Base medium

B.3.1.1 Composition

Enzymatic digest of meat extract	4,3 g
Enzymatic digest of casein	8,6 g
Sodium chloride (NaCl)	2,6 g
Calcium carbonate ($CaCO_3$)	38,7 g
Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	47,8 g
Ox bile for bacteriological use	4,75 g
Brilliant green	9,5 mg
Water	1 000 ml

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B.3.1.2 Preparation

Dissolve the dehydrated basic components or the dehydrated complete medium in the water by boiling for 5 min.

Adjust the pH, if necessary, so that it is 8,2 ± 0,2 at 25 °C.

Thoroughly mix the medium and aseptically dispense in 10 ml amounts into sterile tubes (6.9).

The base medium may be stored for 4 weeks at 3 °C ± 2 °C.

Aseptically add novobiocin to final concentration 50mg/L
iodine 4g/l and potassium iodide 5g/L

Dispense the medium aseptically into sterile flasks (6.9) of suitable capacity to obtain the portions necessary for the test.

The complete medium shall be used the day of its preparation.

Directly suspend approximatively 1 ml of culture into 10 ml MKTTN
Incubation: 24 +/-3 hours at 36-38°C.

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Tetrathionate is produced from thiosulfate by adding iodine to the culture medium.
Tetrathionate suppresses the growth of coliform and other enteric bacteria.
Salmonella, Proteus, and some other species of bacteria can reduce tetrathionate and are not inhibited.
Calcium carbonate buffers the sulfuric acid, which is liberated when tetrathionate is reduced.
Bile promotes the growth of *Salmonella*, but largely inhibits the accompanying bacteria.
Brilliant green and novobiocin primarily inhibit the growth of Gram-positive bacteria.

RVS

- Action :
  - The malachite green and magnesium chloride concentrations of the present culture medium are less than those of the Salmonella Enrichment Broth according to RAPPAPORT in order to improve the growth of Salmonella at 43 °C.
  - Peptone from soymeal is also used for the same reason.
  - Lowering pH to 5.2 increases selectivity.

ALCAIDE et al. (1982) have reported that addition of novobiocin (40 mg/liter) enhances the inhibition of accompanying flora.
Not included in the standard

- Typical Composition (g/liter)
  - Peptone from soymeal 4.5
  - Magnesium chloride hexahydrate 28.6
  - Sodium chloride 7.2; di-potassium hydrogen phosphate 0.18
  - Potassium di-hydrogen phosphate 1.26
  - Malachite-green 0.036.

RVS

Adjust the pH, if necessary, so that after sterilization it is 5,2 ± 0,2.

Before use, dispense into test tubes (6.9) in 10 ml quantities.

Sterilize for 15 min in the autoclave (6.1) set at 115 °C.

Store the prepared medium at 3°C ± 2°C. Use the medium the day of its preparation.

- Experimental Procedure
  - Inoculate the culture medium with the sample or material from a pre-enriched culture (e.g. Peptone Water Buffered) at least at a 1/100 ration (0.1ml in 10ml)
  - incubate for 24+/-3 hours at 41.5 +/- 1°C.
  - streak material from the resulting cultures onto selective culture media.