

Specification

Medium for the detection of coliforms by membrane filtration in water analyses according to ISO 9308-1:2000 standard.

Presentation

	Packaging Details	Shelf Life	Storage
30 Prepared Plates 55 mm Plates for filtration purposes with: 9 ± 1 ml	1 box containing: 5 plastic bags with 6 plates of 55 mm/ bag.	6 months	2-25°C

Composition

Composition (g/l):

Meat peptone.....	10.000
Meat extract.....	5.000
Lactose	20.000
Yeast extract.....	6.000
Bromothymol blue	0.050
Tergitol ® 7.....	0.100
TTC steril solution 1%.....	2.500
Agar.....	15.000

Description /TechniqueDescription:

This medium is formulated for the presumptive identification of coliforms in drinking water, by membrane filtration according to ISO 9308-1:2000.

Technique:

While using the membrane filter technique for the presumptive identification of coliforms in water, it should be kept in mind that the minimum volume to be filtered depends on the type of water being tested. If necessary dilute with sterile phosphate buffer in order to obtain the number of colonies on the membrane appropriate for counting.

For every water sample two volumes must be filtered over two different membranes and incubated on Chapman TTC Agar at 35°C and 44°C respectively.

After 48 hours typical colonies have the appearance as follow:

- *Escherichia coli*, *Citrobacter* spp.: Yellow with a centred orange nucleus under the membrane filter (MF).
- *Klebsiella* spp.: Brick red or yellow without a nucleus. The medium under the (MF) is yellow.
- *Enterobacter* spp.: Dark yellow or brick red with an orange nucleus. The medium is also yellow.
- Non lactose-fermenters: Violet or indigo colonies. The medium turns blue.

Most coliforms can not grow on this medium when incubated at 44°C, except *E. coli* which forms a colony with a characteristic appearance.

Results are always expressed per 100 mL sample including any applied dilutions. Estimation is done by taking typical colonies which have grown at 35°C as faecal coliforms, together with those grown at 44°C as *E. coli*. Nevertheless, according to legislation and despite the medium's selectivity, results can only be considered as presumptive and all coliform colonies have to be confirmed by following the criteria below:

Typical appearance in EMB Agar or Endo Agar Base and characteristic reactions in Kligler Iron Agar.

For the confirmation of faecal *E. coli*, the following characteristics are used for verification: a motile, Gram negative bacillus and lactose fermenter with acid and gas production, which gives negative results on the citrate test and indol production positive.

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

Note: Incubation times greater than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications.

After incubation, enumerate the yellowish colonies as pressumptive *E.coli* or any other coliform.

Calculate total microbial count per ml of sample by multiplying the average number of colonies per membrane by the inverse dilution factor. Report results as Colony Forming Unit (CFU's) per ml along with incubation time and temperature.

Confirmation of *E.coli* detection is required with further microbiological or biochemical tests.

Quality control**Physical/Chemical control**

Color : Green

pH: 7.2 ± 0.1 at 25°C**Microbiological control**Membrane Filtration /Practical range 100 ± 20 CFU; Min. 50 CFU (Productivity)./ 10^4 - 10^6 CFU for Selectivity.

Microbiological control according to ISO 11133:2014/ Adm 1:2018.

Aerobiosis. Incubation at $36 \pm 2^\circ\text{C}$, reading at 21 ± 3 h**Microorganism***Escherichia coli* ATCC® 25922, WDCM 00013*Escherichia coli* ATCC® 8739, WDCM 00012*Citrobacter freundii* ATCC® 43864, WDCM 00006*Enterococcus faecalis* ATCC® 19433, WDCM 00009*Ps. aeruginosa* ATCC® 9027, WDCM 00026*Escherichia coli* ATCC® 11775, WDCM 00090*E. coli* NCTC® 13167, WDCM 00179**Growth**Good ($\geq 50\%$) Colonies Yellow-orange under MF.Good ($\geq 50\%$) Colonies Yellow-orange under MF.Good ($\geq 50\%$) Colonies Yellow-orange under MF.

Partial Inhibition

Good- Red colonies w. blue center.

Good ($\geq 50\%$) Colonies Yellow-orange under MF.Good ($\geq 50\%$) Colonies Yellow-orange under MF.**Sterility Control**

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

Check at 7 days after incubation in same conditions

Bibliography

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- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. APHA. Washington.
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- ISO 9308-1:2000 Standard. Water Quality - Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method.
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