Mac Conkey/Agar
(Lactose without crystal violet)

356-4154

DEFINITION
Medium used for the detection, isolation and enumeration of coliforms in food and water.

STANDARDS
WATER
- **NF EN ISO 10705-1 (October 2001):** Water quality - Detection and enumeration of Bacteriophagia - Part 1: Enumeration of ARN F specific Bacteriophagia (IC: T90-440-1).

- **NF EN ISO 10705-2 (October 2001):** Water quality - Detection and enumeration of Bacteriophagia - Part 2: Enumeration of somatic Coliphagia (IC: T90-440-2).

PRINCIPLE
The principle of the medium relies on the ability of coliforms to ferment lactose (red colonies).

PRESENTATION
Dehydrated
500 g **code 356-4154**

STORAGE
- +15℃ to 25℃, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	20 g
Lactose	10 g
Bile salts	5 g
Sodium chloride	5 g
Neutral red	30 mg
Agar	12 g
Distilled water	1,000 ml

Final pH (25℃) = 7.4 ± 0.2

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 125 ml Pyrex bottles with autoclave-proof stopper
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Thermostatically-controlled incubator or incubation room, precise to ± 1℃
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 52 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix until a homogenous suspension is obtained.
Heat gently, swirling frequently, then bring to the boil until completely dissolved. If necessary, adjust the pH to 7.4 ± 0.2. Dispense, then sterilize in autoclave at 121℃ ± 1℃ for 15 minutes.
This medium can be used non-autoclaved on the day of its preparation ; in this case it should be boiled for 2 or 3 minutes.

Reconstitution ratio : 52 g/l.
500 g of powder makes 9.6 liters of medium.

PROTOCOL
• Inoculation
This medium is used in certain countries for enumeration of coliforms (*E. coli, Citrobacter, Klebsiella, Enterobacter*) in food products. Using sterile pipettes, place 1 ml of product to be examined, or its different dilutions, in empty, sterile Petri dishes. Quickly pour a sufficient quantity (15 - 20 ml) of medium, previously cooled to 44℃ - 47℃, and mix thoroughly.
Leave to cool on a cold level surface. Once it has completely solidified, pour a second layer of virgin medium over the top of the first layer; it should be about 2 mm thick. Leave to cool again.

• Incubation
After complete gelification, turn the dishes over and incubate in this position at 36℃ ± 2℃ for 20 ± 4 hours (variations possible according to the standard followed).

READING AND INTERPRETATION
Following appropriate incubation, enumerate any brick-red colonies (coliforms) with a diameter of at least 0.5 mm.
Transfer the result to the unit of product examined (1 ml or 1 g).

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PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24h culture at 37°C
Escherichia coli ATCC 25922	Good growth Pink to red colonies
Salmonella enteritidis ATCC 13076	Good growth Colorless colonies
Shigella flexneri ATCC 12022	Good growth Colorless colonies
Staphylococcus aureus ATCC 25923	Good growth Small pink to red colonies
Staphylococcus epidermidis ATCC 12228	Good growth Small pink to red colonies
Enterobacter aerogenes ATCC 13048	Good growth Pink to red colonies

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Mac Conkey without crystal violet / Coliforms / Water / Detection / Enumeration / Lactose / Medium.

BIBLIOGRAPHY

- **International Standards for Drinking Water, 2nd Ed.** World Health Organization, Geneva (1958).
- **The Bacteriological Examination of Water Supplies, 4th Ed.,** Churchill Ltd., London, (1958).

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