

Recommended as a differential medium for the selection and recovery of the Enterobacteriaceae and related enteric gramnegative bacilli.

|                                  |                      |                |          |
|----------------------------------|----------------------|----------------|----------|
| REF: V/MA1.100<br>REF: V/MA1.250 | 100 Gram<br>250 Gram | REF: V/MA1.500 | 500 Gram |
|----------------------------------|----------------------|----------------|----------|

## CLINICAL SIGNIFICANCE

The MacConkey media are well known and popular enrichment system for coliform bacteria. MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (1,2). Original formulation of MacConkey included ox bile as inhibitor of gram-positive bacteria and litmus as the indicator of the acid production from lactose. Later litmus was substituted by phenol red indicator to make interpretation easier and more precise. The most significant modification to the original formulation is the substitution of ox bile by purified bile salts that improve the selectivity and avoid the inherent turbidity which is due to the fat material of the bile. Another modification was the inclusion of supplementary inhibitors such as crystal violet and pH indicator neutral red. MacConkey Agar has been recommended for use in microbiological examination of foodstuffs (3) and for direct plating of water samples for coliform counts (4). These media are also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5).

## METHOD PRINCIPLE

Original medium contains protein, bile salts, sodium chloride and two dyes. Omission of sodium chloride from the medium prevents the spreading of Proteus colonies (6). The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose-fermenting strains grow as red or pink coloured colonies and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as Shigella and Salmonella are colourless and transparent and typically do not alter appearance of the medium.

## MEDIA COMPOSITION

| Ingredient      | Concentration (g/l) |
|-----------------|---------------------|
| Peptone         | 1.5                 |
| Tryptone        | 1.5                 |
| Gelatin peptone | 17                  |
| Lactose         | 10                  |
| Bile salts      | 1.5                 |
| Crystal violet  | 0.001               |
| Neutral red     | 0.03                |
| Agar            | 15                  |

**Final pH 7.1 ± 0.2 at 25°C.**

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

**S56:** dispose of this material and its container at hazardous or special waste collection point.

**S57:** use appropriate container to avoid environmental contamination.

**S61:** avoid release in environment.

For further information, refer to the MacConkey Agar without salt material safety data sheet.

## STORAGE AND STABILITY

**Lab.Vie.** MacConkey Agar without salt should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Deterioration

**Lab.Vie.** MacConkey Agar without salt is Light yellow to light pink homogeneous free flowing powder. Prepared medium is Orange red coloured clear to slightly opalescent gel forms in Petri plates. If there are any physical changes, discard the medium. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations

## SPECIMEN COLLECTION AND PRESERVATION

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,9). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4). After use, contaminated materials must be sterilized by autoclaving before discarding.

## TYPE OF SPECIMEN

Clinical - faeces, urine, Foodstuffs and Dairy samples, Water samples

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile tubes
- Incubator
- Autoclave

## PROCEDURE

1. Suspend 18 grams in 950 ml purified / distilled water.
2. Adjust pH to 6.8 ± 0.2 at 25°C.
3. Heat if necessary to dissolve the medium completely.
4. Dispense in 95 ml amounts into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
5. Cool to about 50-55°C and add aseptically 5 ml sterile 40% Urea solution per 95 ml basal medium.
6. Mix well and dispense into sterile test tubes. Allow to cool in an upright position.

Do not overheat or reheat the medium as urea decomposes very easily.

## PERFORMANCE CHARACTERISTICS

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact **Lab.Vie.** Technical support.

## RESULTS AND INTERPRETATION

| Organisms                                   | Urease                           | Indole                                                     | Motility                                                             |
|---------------------------------------------|----------------------------------|------------------------------------------------------------|----------------------------------------------------------------------|
| <i>Escherichia coli</i><br>ATCC 25922       | Negative reaction, no change     | Positive reaction, red ring at the interface of the medium | Positive, growth away from stabline causing turbidity                |
| <i>Klebsiella pneumoniae</i><br>ATCC 13883  | Weakly positive                  | Negative reaction no colour development / cloudy ring      | Negative growth along the stabline, surrounding medium remains clear |
| <i>Proteus mirabilis</i><br>ATCC 25933      | Positive reaction, cerise colour | Negative reaction no colour development / cloudy ring      | Positive, growth away from stabline causing turbidity                |
| <i>Proteus vulgaris</i><br>ATCC 13315       | Positive reaction, cerise colour | Positive reaction, red ring at the interface of the medium | Positive, growth away from stabline causing turbidity                |
| <i>Salmonella typhimurium</i><br>ATCC 14028 | Negative reaction, no change     | Negative reaction no colour development / cloudy ring      | Positive, growth away from stabline causing turbidity                |

## REFERENCES

1. Rustigian and Stuart (1941) Proc. Soc. Exp. Biol. Med., 47:108.
2. McFaddin J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
3. Ewing (1986) Edwards and Ewings 'Identification of Enterobacteriaceae', 4th ed. Elsevier Science Publishing Co., Inc., New York. Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.