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MacConkey II Agar

PD032; MACCONKEY AGAR; 90 mm Petri dish with media

Appearance: reddish-purple, slightly opalescent

MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

Intended Use - MacConkey Agar is recommended for use with clinical specimens likely to contain mixed microbial flora, such as urine, respiratory and wound, because it allows a preliminary grouping of enteric and other gram-negative bacteria. MacConkey Agar is also used in the BAM (Bacteriological Analytical Manual) of the Food and Drug Administration (FDA) procedure for *isolating E. coli* from foods. It was specially designed to improve the inhibition of swarming Proteus species, to achieve more definitive differentiation of lactose fermenters and non-fermenters, and for the promotion of superior growth of enteric pathogens.

Principle - MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey. The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms. MacConkey Agar, CS ("Controlled Swarming") contains carefully selected raw materials to reduce swarming of Proteus spp., which could cause difficulty in isolating and enumerating other Gram-negative bacilli. . It is only slightly selective since the concentration of bile salts, which inhibits gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Formula / Liter – Pancreatic Digest of Gelatin 17 g, Pancreatic Digest of Casein 1.5 g, Digest of Animal Tissue 1.5 g, Lactose 10 g , Bile Salts 1.5 g, Sodium Chloride 5 g, Neutral Red 0.03 g, Crystal Violet. 0.001 g, Agar 13.5 g

Physical Appearance - Dark pink-purple, and trace to slightly hazy

Storage – 2 – 25 °C

pH at RT – 7.1 ± 0.2

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Productivity Test (from 10-100 cfu)		
Strains	**Lactose Fermentation**	**Recovery**
E. coli ATCC 8739	Positive: Pink colonies	≥ 0.5
Proteus mirabilis ATCC 12453	Negative:Colorless, no swarming	≥ 0.5
Salmonella typhymurium ATCC 14028	Negative: Colorless with black shadow colonies	≥ 0.5
Pseudomonas aeruginosa ATCC 9027	Negative: Pink to green	≥ 0.5
Shigella sonnei ATCC 29930	Negative: Colorless to slight pink	≥ 0.5
Selectivity (10^4 cfu)		
Enterococcus faecalis ATCC 29212	Inhibited	

Warning and Precautions - If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions” and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding. Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use.

Procedures- Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate plates, protected from light, at 35 ± 2 °C (do not use CO2-enriched atmosphere with MacConkey II Agar) or other appropriate temperature for 18–24 h

Procedures Limitations - Not all strains of *E. coli* ferment lactose. For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens