

MacConkey Agar EP/USP/ISO

Cat. 1052

For the isolation and identification of Enterobacteriaceae from feces, urine wastewater and foods.

Practical information

Applications	Categories
Selective isolation	Enterobacteria
Selective isolation	Escherichia coli
Detection	Shigella

Industry: Water / Pharmaceutical/Veterinary / Cosmetics / Clinical / Food / Final product Quality Control

Regulations: USP / European Pharmacopoeia / ISO 21150 / ISO 21567



Principles and uses

MacConkey Agar is used for the selective isolation and identification of Enterobacteriaceae from feces, urine, wastewater and foods. It is also a selective and differential medium for the isolation of enteric Gram-negative bacteria.

The specimen can be streaked directly on the medium or inoculated first into an enrichment broth such as a Tetrathionate Broth (Cat. 1114), Selenite Cystine Broth (Cat. 1220), GN Enrichment Broth (Cat. 1248) or MacConkey Broth (Cat. 1210).

The European Pharmacopoeia, USP recommends this media in the paragraph 2.6.13: "Microbiological examination of non-sterile products: Test for specified microorganisms" for the growth promoting and indicative properties of the media in the test for E. coli. Also, this medium is recommended for the testing of E. coli in products.

ISO 21567 recommends this medium for the detection of Shigella spp. It is used to obtain isolated colonies. After incubating the plates of Agar McConkey a confirmatory step is necessary.

ISO 21150 recommends this medium for the detection of E. coli in cosmetic products.

Pancreatic digest of gelatin and peptones meat & casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bile salts and crystal violet are the selective agents, and inhibit Gram-positive organisms. Neutral red is the pH indicator. When lactose is fermented, the pH of the medium decreases, changing the color of neutral red to pink. Bacteriological agar is the solidifying agent.

Isolated colonies of Escherichia coli are brick red in color and are surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation.

Formula in g/L

Bacteriological agar	13,5	Bile salts	1,5
Crystal violet	0,001	Gelatin pancreatic digest	17
Neutral red	0,03	Peptone mixture	3
Sodium chloride	5	Lactose monohydrate	10

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 50 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 47 °C, mix well and dispense into plates. Allow the plates to solidify and place them upside down to avoid excessive moisture on the surface of the medium.

Instructions for use

» For clinical diagnosis, the type of samples are urine and feces.

- Spread a plate with loop or swab
- Incubate in aerobic conditions at 35 ± 2 °C for 18-24 hours.
- Reading and interpretation of results.

» For other uses not covered by the CE marking:

Test of specified microorganisms (Enterobacteriaceae) according to European Pharmacopoeia:

- Inoculate and incubate at 30-35 °C for 18-24 h in Trypticasein Soy Broth (TSB) (Cat. 1224).
- Subculture in MacConkey Broth and incubate at 42-44 °C for 24-48 h (Cat. 1210)
- Streak onto a plate of MacConkey Agar.
- Incubate at 30-35 °C for 18-72 hours.
- Growth of colonies with precipitated bile indicates the possible presence of E.coli.
- This is confirmed by identification test.
- The products complies with the test if no colonies are present or if the identification test are negative.

Detection of Shigella spp according to ISO 21567:

- Inoculate the sample in Shigella Broth (Cat. 2078) with 0,5 µg / mL of novobiocin. Incubate in anaerobiosis conditions at $41,5\pm 1$ °C for 16-20 hours.
- From shigella broth, inoculate MacConkey agar (Cat. 1052), XLD Agar (Cat. 1274) and Hektoen Enteric Agar (Cat. 1030). Incubate at a temperature of 37 ± 1 °C for 20-24 hours.
- Biochemical test must be performed from suspicious and characteristics colonies.

Detection of E. coli according to ISO 21150:

- Disperse 1 g or 1 ml of the product in at least 9 ml of the broth enrichment Broth Eugon LT 100 (Cat. 2110). According to the indications of the ISO, it can be used differently depending on whether miscible or immiscible products are analyzed or if samples are filterable products. Incubate at $32,5\pm 2,5$ °C for at least 20 hours (maximum 72 h).
- From the enrichment broth, MacConkey agar is inoculated to obtain isolated colonies. Incubate at $32,5\pm 2,5$ °C for at least 24 hours (maximum 48 h).
- Confirmation is made with suspicious colonies.

It is recommended to streak samples onto other selective media such as Eosin Methylene Blue Agar (Cat. 1039), SS Agar (Cat. 1064), XLD Agar (Cat. 1080), Hektoen Enteric Agar (Cat. 1030), Bismuth Sulfite Agar (Cat. 1011), especially for Salmonella typhi, and/or Brilliant Green Agar (Cat. 1078), especially for Salmonella. See the listings in this manual for these formulations.

CHARACTERISTICS OF COLONIES:

Escherichia coli: Red or Pink; Not mucoid; Round; Opaque precipitate of bile salts.

Klebsiella: Large, Red, Mucoid .

Enterobacter aerogenes: Pink to Red.

Serratia: Red to Pink, Not Mucoid.

Arizona and Citrobacter: Colorless, transparent; Red if lactose is fermented.

Proteus: Colorless and transparent. Yellow color around the colony.

Salmonella: Colorless, transparent or amber. Yellow color around the colony.

Shigella sonnei*: Colorless to pale pink, translucent, lactose negative

Shigella, other species: Colorless, translucent, lactose negative.

*Shigella sonnei can ferment lactose after more than 40 hours of incubation, which gives a weak reaction similar to Escherichia coli after the same time.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige-pink	Violet-red.	7,1±0,2

Microbiological test

According to European Pharmacopoeia; Escherichia coli ATCC 8739:

Incubation conditions: (30-35 °C / 18-72 h)

Inoculation conditions: (<=100 CFU)

According to ISO 21567; Shigella dysenteriae:

Incubation conditions:(37 ± 1 °C / 20-24 h)

According to ISO 21150; Escherichia coli ATCC 8739:

Incubation conditions: (30-35 °C / 24-48 h)

Rest of strains; Enterobacter aerogenes, Proteus vulgaris, Salmonella enteritidis and Staphylococcus aureus, Escherichia coli ATCC 25922:

Incubation conditions: (35 ± 2 °C / 24 h).

Microorganisms	Specification	Characteristic reaction
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Klebsiella aerogenes ATCC 13048	Good growth	Pink-red colonies
Salmonella enteritidis ATCC 13076	Good growth	Colorless colonies
Shigella dysenteriae ATCC 13313	Good growth	Colorless colonies
Proteus vulgaris ATCC 13315	Good growth	Colorless colonies
Escherichia coli ATCC 25922	Good growth	Pink-red (bile precipitate) colonies
Staphylococcus aureus ATCC 6538	Inhibition	
Escherichia coli ATCC 8739	Good growth	Pink-red (bile precipitate) colonies

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

MacConkey J. H. 5:33. 1905. Joseph Md. State. Dept. Health. Procedures, 1960.
Harmonized European Pharmacopoeia

ISO 21567. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Shigella spp.

ISO 21150. Cosmetics Microbiology. Detection of Escherichia coli