

MACCONKEY AGAR

AGMC-IEP-500

- **Principle**

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

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 gelatine and peptones meat and 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 colour of neutral red to pink. Bacteriological agar is the solidifying agent.

Isolated colonies of *Escherichia coli* are brick red in colour 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.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 21150 and ISO 21567 as well as the USP and European Pharmacopoeia reference methodology.

- **Composition**

Ingredients	g/L
Bacteriological agar	13.50
Crystal violet	0.001
Neutral red	0.03
Sodium chloride	5.00
Bile salts	1.50
Gelatine pancreatic digest	17.00
Peptone mixture	3.00
Lactose monohydrate	10.00

- **Preparation**

Suspend 50 grams of the medium in one litre 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.

- **Applications and use**

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

- 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) (STRB-0EP-500).
- Subculture in MacConkey Broth and incubate at 42-44 °C for 24-48 h.
- 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 comply with the test if no colonies are present or if the identification test is negative.

Detection of *Shigella* spp according to ISO 21567:

- Inoculate the sample in Shigella Broth with 0.5 µg / mL of novobiocin. Incubate in anaerobiosis conditions at 41.5±1 °C for 16-20 hours.
- From shigella broth, inoculate MacConkey agar (AGMC-IEP-500), XLD Agar (AGXL-00I-500) and Hektoen Enteric Agar (HEAG-00P-500). 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. According to the indications of the ISO, it can be used differently depending on whether miscible or immiscible products are analysed or if samples are filterable products. Incubate at 32.5±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±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 (EMBA-00I-500), SS Agar (SSAG-IEP-500), XLD Agar (AGXL-00I-500), Hektoen Enteric Agar (HEAG-00P-500), especially for *Salmonella typhi*, and/or Brilliant Green Agar (BGAG-00I-500), 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* spp: Large, Red, Mucoid. *Enterobacter aerogenes*: Pink to Red. *Serratia* spp: Red to Pink, Not Mucoid. *Arizona* spp and *Citrobacter* spp: Colourless, transparent; Red if lactose is fermented. *Proteus*: Colourless and transparent. Yellow colour around the colony. *Salmonella* spp: Colourless, transparent or amber. Yellow colour around the colony. *Shigella sonnei**: Colourless to pale pink, translucent, lactose negative *Shigella*, other species: Colourless, 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	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Beige-pink
Colour of the prepared medium	Violet-red
Final pH (25 °C)	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	ATCC	Growth	Characteristic reaction
<i>Klebsiella aerogenes</i>	13048	Good growth	Pink-red colonies
<i>Salmonella enteritidis</i>	13076	Good growth	Colourless colonies
<i>Shigella dysenteriae</i>	13313	Good growth	Colourless colonies
<i>Proteus vulgaris</i>	13315	Good growth	Colourless colonies
<i>Escherichia coli</i>	25922	Good growth	Pink-red (bile precipitate) colonies
<i>Staphylococcus aureus</i>	6538	Inhibition	-
<i>Escherichia coli</i>	8739	Good growth	Pink-red (bile precipitate) colonies

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **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

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.