

## TECHNICAL DATA SHEET

# MACCONKEY AGAR

### DETECTION OF *ENTEROBACTERIACEAE*

## 1 INTENDED USE

MacConkey agar is a selective medium for the isolation of enterobacteria in water, food, pharmaceutical products and biological samples of animal origin and in cosmetic products. .

The typical composition corresponds to that defined in the EU Pharmacopeia and in the standards NF EN ISO 21567 and NF EN ISO 21150.

## 2 HISTORY

The formulation of MacConkey for the isolation of enterobacteria has been modified a number of times since its inception. The present medium is the "classical" formula used for many years by authors such as Block and Ferguson, who found the medium satisfactory for the isolation of fastidious *Shigella*.

## 3 PRINCIPLES

Bile salts and crystal violet inhibit the growth of Gram positive bacteria. The dye inhibits primarily the development of enterococci and staphylococci.

The fermentation of lactose to acid is revealed in presence of neutral red by the formation of red or pink colonies.

Lactose-negative colonies form colorless colonies.

## 4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

|                                     |         |
|-------------------------------------|---------|
| - Pancreatic digest of gelatin..... | 17,0 g  |
| - Tryptone.....                     | 1,5 g   |
| - Peptic digest of meat.....        | 1,5 g   |
| - Lactose.....                      | 10,0 g  |
| - Bile salts.....                   | 1,5 g   |
| - Sodium chloride.....              | 5,0 g   |
| - Neutral red.....                  | 30,0 mg |
| - Crystal violet.....               | 1,0 mg  |
| - Bacteriological agar.....         | 13,5 g  |

pH of the ready-to-use media at 25 °C : 7,1 ± 0,2.

## 5 PREPARATION

### Preparation of dehydrated media :

- Dissolve 50,0 g of dehydrated media (BK050) in 1 liter distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into Petri plates and let solidify on a cold, flat surface.

✓ **Reconstitution :**  
50,0 g/L

✓ **Sterilization :**  
15 min at 121 °C

### Use of the ready-to-melt media :

- Melt the media prepared in advance or the base medium (BM180) for the minimum amount of time necessary in order to achieve total liquefaction.
- Cool and maintain in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.

## 6 INSTRUCTIONS FOR USE

### Detection of *Escherichia coli* (harmonized chapters of the Pharmacopieae)

- Dry the plates in an incubator, covers partially removed.
- Inoculate the enrichment broth (MacConkey broth) onto a plates prepared as above.
- Incubate at 30-35 °C for 18 to 72 hours.
- The presence of colonies on the plates requires the completion of the confirmation tests for *Escherichia coli*.

✓ **Inoculation :**  
Surface streaking

✓ **Incubation :**  
18 to 72 h at 30-35 °C

### Detection of *Escherichia coli* (Cosmetics, NF EN ISO 21150)

- Dry the plates in an incubator, covers partially removed.
- Inoculate the enrichment broth (Eugon LT100 broth) onto a plates prepared as above.
- Incubate at 30-35 °C for 24 to 48 hours.
- The presence of brick-red colonies, with or without a zone of precipitated bile, requires the completion of confirmation tests for *Escherichia coli* : Gram stain, growth on EMB agar (BK056).

✓ **Inoculation :**  
Surface streaking

✓ **Incubation :**  
24 to 48 h at 30-35 °C

### Detection of *Shigella* spp (Food microbiology, NF EN ISO 21567)

- Dry the plates in an incubator, covers partially removed.
- Inoculate the enrichment broth (for *Shigella*) onto a plates prepared as above and onto other recommended agars : Hektöen agar (BK067) and XLD agar (BK058).
- Incubate at 37 ± 1 °C for 20 to 24 hours.
- If characteristic colonies are present, proceed with the necessary confirmation tests.

✓ **Inoculation :**  
Surface streaking

✓ **Incubation :**  
20 to 24 h at 37°C

## 7 RESULTS

Lactose-positive colonies are red and are surrounded by a halo of precipitated bile salts. Lactose-negative colonies are colorless.

*Escherichia coli* present brick-red colonies, with or without zones of precipitated bile.

*Shigella sonnei* colonies are colorless to pale pink and translucent. Other *Shigella* spp. are colorless and translucent.

## 8 QUALITY CONTROL

**Dehydrated media** : rose-beige powder, free-flowing and homogeneous.

**Prepared media** : red-violet agar.

Typical culture response after 18 hours of incubation at 30-35 °C :

| Microorganisms                     | Growth Productivity Ratio $P_R$ | Characteristics |
|------------------------------------|---------------------------------|-----------------|
| <i>Escherichia coli</i> WDCM 00012 | $P_R \geq 50 \%$                | Red colonies    |

Typical culture response after 24 hours of incubation at 37 °C :

| Microorganisms         |            | Growth | Characteristics    |
|------------------------|------------|--------|--------------------|
| <i>Shigella sonnei</i> | WDCM 00127 | Good   | Colorless colonies |

## 9 STORAGE / SHELF LIFE

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**Dehydrated media :** 2-30 °C.

**Ready-to-melt media in vials :** 2-8 °C.

The expiration dates are indicated on the labels.

**Prepared media in vials (\*) :** 180 days at 2-8 °C.

**Prepared media in plates (\*) :** 30 days at 2-8 °C.

(\*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 10 PACKAGING

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**Dehydrated media :**

500 g bottle ..... BK050HA

**Ready-to-melt media :**

10 x 100 mL vials ..... BM18008

## 11 BIBLIOGRAPHY

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MacConkey. 1905. Lactose-fermenting bacteria in faeces. J. Hyg., 8: 333-379.

NF EN ISO 21567. Mars 2005. Microbiologie des aliments. Méthode horizontale pour la recherche de *Shigella* spp..

NF EN ISO 21150. Septembre 2009. Cosmétiques. Microbiologie. Détection d'*Escherichia coli*.

Pharmacopée Européenne. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.

## 12 ADDITIONAL INFORMATION

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The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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