TECHNICAL DATA SHEET

CETRIMIDE AGAR

DETECTION AND ENUMERATION OF *PSEUDOMONAS AERUGINOSA*

1 INTENDED USE

Cetrimide Agar is a selective medium for the isolation and enumeration of *Pseudomonas aeruginosa* in biological samples of animal origin and in pharmaceutical and cosmetic products.
Its typical composition corresponds to that defined in European (EP), United States (UP) and Japanese (JP) Pharmacopeias, and in the Directive NF EN ISO 22717 for the control of cosmetic products.

2 HISTORY

The formula of this medium was derived from the King A medium, favoring pyocyanin production by *Pseudomonas aeruginosa*. In 1951, Lowbury recommended the use of cetrimide in a selective medium for the isolation of *Pseudomonas*. The concentration of the inhibitor was reduced by Lowbury and Collins (1955) as a result of its improved purity.

3 PRINCIPLES

Cetrimide (cetyltrimethylammonium bromide) is a quaternary ammonium compound which inhibits a large number of bacteria including species of *Pseudomonas* other than *Pseudomonas aeruginosa.*

The production of pyocyanin (a blue, non-fluorescent pigment soluble in water and chloroform) is stimulated by magnesium chloride and potassium sulfate.

The medium also favors the production of fluorescent pigments (pyoverdins) by some strains of *Pseudomonas aeruginosa*.

Most *Pseudomonas aeruginosa* can be identified by the characteristic grapelike odor of aminoacetophenone.

4 TYPICAL COMPOSITION

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

For 1 liter of optimized media:

- Pancreatic digest of gelatin ..... 20.0 g
- Glycerol ..... 10 mL
- Cetrimide ..... 0.3 g
- Magnesium chloride ..... 1.4 g
- Sulfate de potassium ..... 10.0 g
- Bacteriological agar ..... 13.6 g

pH of the media ready-to-use at 25 °C: 7.2 ± 0.2.

For 45.3 g of dehydrated base BK049

- Pancreatic digest of gelatin ..... 20.0 g
- Cetrimide ..... 0.3 g
- Magnesium chloride ..... 1.4 g
- Potassium sulfate ..... 10.0 g
- Bacteriological agar ..... 13.6 g

Glycerol not supplied.

For 1 liter of ready-to-melt media (BM184)

- Pancreatic digest of gelatin ..... 20.0 g
- Glycerol ..... 10 mL
- Cetrimide ..... 0.3 g
- Magnesium chloride ..... 1.4 g
- Potassium sulfate ..... 10.0 g
- Bacteriological agar ..... 13.6 g

5 PREPARATION

- Suspend 45.3 g of dehydrated media (BK049) in 1 liter of distilled or deionized water.
- Add 10 mL of glycerol.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the medium at 44-47°C.
- Pour into sterile Petri dishes and let solidify on a cold surface.

✓ **Reconstitution:**
45.3 g/L
+ 10 mL of glycerol

✓ **Sterilization:**
15 min at 121 °C

Use of ready-to-melt media:

- Melt the media (if it was prepared in advance) or use the ready-to-melt media containing glycerol (BM184), for the minimum amount of time necessary to achieve total liquefaction.
- Cool and maintain at 44-47 °C.

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator with the covers partially removed.
- Inoculate by streaking with the aid of a sterile loop onto the surface of the agar.
- Incubate at 30-35 °C
  - for 24 to 48 hours, as defined in the Directive NF EN ISO 22717 for cosmetic products.
  - for 18 to 72 hours following the Pharmacopoeia, in the case of pharmaceutical products.

✓ **Inoculation:**
Surface streaking

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

7 RESULTS

Pseudomonas aeruginosa can present the following aspects:

- characteristic yellow-green pigmentation and a fluorescence under UV light at 254 nm.
- grayish, mucoid colonies, with or without pigmentation.

Note:
Occasionally, strains of *Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia, Alcaligenes* and *Aeromonas* may also grow, causing a slight yellowing of the medium. This color is easily differentiated from fluorescein production since the former does not fluoresce.

8 QUALITY CONTROL

Dehydrated media: cream-white powder, free-flowing and homogeneous.
Complete prepared media: whitish agar.

Typical culture response (NF EN ISO 4793, EP, USP & JP):

Microorganisms		Theoretical growth (P_R: Productivity Ratio)
Pseudomonas aeruginosa	WDCM 00026	$P_R \geq 70$ % [1]
Escherichia coli	WDCM 00012	Inhibited [2]

[1] after 16 to 18 hours of incubation at 32.5°C, inoculum ≤ 10^2 microorganisms
[2] after 72 hours of incubation at 32.5°C, inoculum ≥ 10^2 microorganisms

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
Media ready-to-melt in vials: 2-8 °C.
The expiration dates are indicated on the labels.

Complete, prepared media in vials (*): 180 days at 2-8 °C.
Complete, prepared media in plates (*): 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated media (base):
500 g bottle..... BK049HA

Ready-to-melt media:
10 x 100 mL..... BM18408

11 BIBLIOGRAPHY

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Brown, V.I., and Lowbury, E.J.L. 1965. Use of an improved Cetrimide Agar Medium and of culture methods for *Pseudomonas aeruginosa*. J. Clin. Pathol.,18: 752.

NF EN ISO 22717. February 2016. Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa.

NF EN ISO 4973. September 2023. Cosmetics — Microbiology — Quality control of culture media and diluents used in cosmetics standards.

European Pharmacopeia. Chapiter 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonized method.

The United States. Chapter <62> Microbiological examination of non-sterile products: Test for specified products.

The Japanese Pharmacopoeia. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products.

12 ADDITIONAL INFORMATION

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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