TECHNICAL DATA SHEET

CN AGAR FOR *PSEUDOMONAS*

ENUMERATION OF *PSEUDOMONAS AERUGINOSA* IN WATER

1 INTENDED USE

CN Agar for *Pseudomonas* is a selective medium for the isolation and enumeration of *Pseudomonas aeruginosa* in bottled water, swimming pools and water destined for human consumption.

The typical composition of the media responds to the formulation described in the Directives NF EN ISO 16266 and NF T90-421 for water analysis.

2 HISTORY

The formula of the base medium is a modification of King A medium, in which magnesium chloride and potassium sulfate favor pyocyanin production. In 1951, Lowbury recommended the use of cetrimide in a selective medium for the isolation of *Pseudomonas*. Following the improvement in the purity of the inhibiting agent, its concentration was reduced by Lowbury and Collns in 1955. Goto and Enomoto demonstrated that the addition of nalidixic acid, with a decrease in the concentration of cetrimide, allowed for a better recovery of *Pseudomonas aeruginosa* with a correlative increase in pigment production, while contaminating flora (*Proteus*, *Klebsiella*, *Providencia*) were strongly inhibited.

3 PRINCIPLES

Pancreatic digest of gelatin and acid hydrolyzed casein are the nutrient substrates required for the rapid multiplication of *Pseudomonas*.

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

Contaminating yeasts are inhibited by cetrimide.

Nalidixic acid blocks the DNA replication of bacteria sensitive to this antibacterial agent.

Colonies demonstrating a blue-green pigmentation are considered as *Pseudomonas aeruginosa*.
The other types of colonies are presumptive *Pseudomonas aeruginosa* and must be confirmed.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of complete media :

- Pancreatic digest of gelatin ..... 16,0 g
- Acid hydrolysate of casein ..... 10,0 g
- Glycerol ..... 10,0 mL
- Potassium sulfate ..... 10,0 g
- Magnesium chloride ..... 1,4 g
- Cetrimide ..... 0,2 g
- Nalidixic acid ..... 15,0 mg
- Bacteriological agar ..... 11,0 g

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

For 48,6 g of dehydrated base BK165

- Pancreatic digest of gelatin.....16,0 g
- Acid hydrolysate of casein .....10,0 g
- Potassium sulfate.....10,0 g
- Magnesium chloride.....1,4 g
- Cetrimide.....0,2 g
- Nalidixic acid .....15,0 mg
- Bacteriological agar .....11,0 g

Glycerol not furnished

For 1 liter of pre-poured media (BM145)

- Pancreatic digest of gelatin.....16,0 g
- Acid hydrolysate of casein .....10,0 g
- **Glycerol..... 10 mL**
- Potassium sulfate.....10,0 g
- Magnesium chloride.....1,4 g
- Cetrimide.....0,2 g
- Nalidixic acid .....15,0 mg
- Bacteriological agar .....11,0 g

5 PREPARATION

- Suspend 48.6 g of dehydrated base medium (BK165) 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 into tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47°C.
- Pour into sterile Petri dishes (Ø 55 mm) so that the thickness of the agar should be approximately 5 mm.
- Let solidify on a cold surface.

✓ **Reconstitution :**
48,6 g/L
10 mL/L of glycerol

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

6 INSTRUCTIONS FOR USE

- Do not dry the plates.
- Aseptically filter through the appropriate membrane at 0.45 µm a determined volume of the test sample.
- To the surface of plates prepared as above, or using ready-to-use plates (BM145; BM196) brought to room temperature, deposit the membrane on the surface of the agar, filtered side up and making sure that the membrane and agar are in close contact.
- Incubate at 36 ± 2 °C for 22 ± 2 hours and 44 ± 4 hours.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
22 h and 44 h at 36 ± 2 °C

7 RESULTS

Only count plates containing fewer than 150 colonies.
Colonies producing a blue-green (pyocyanin) pigmentation, are considered as confirmed *Pseudomonas aeruginosa*.
Others considered as presumptive *Pseudomonas aeruginosa* :

- Colonies not producing pyocyanin but demonstrating a fluorescence under UV light at 360 nm.
- Colonies demonstrating a red-brown pigmentation without fluorescence.

Presumptive colonies should be submitted to confirmation tests such as ammonia production from acetamide, oxidase production or fluorescence of King B agar.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base media : cream-white powder, free-flowing and homogeneous.
Prepared (complete) media : whitish agar.

Typical cultural response on complete medium after 44 hours of incubation at 36 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio P_R)
Pseudomonas aeruginosa	WDCM 00024	$P_R \geq 70$ %
Pseudomonas aeruginosa	WDCM 00026	$P_R \geq 70$ %
Escherichia coli	WDCM 00013	Inhibited, score 0
Enterococcus faecalis	WDCM 00087	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated base media (without glycerol) : 2-30 °C.
Pre-poured media in Petri plates : 2-8 °C.
The expiration dates are indicated on the labels.

Base media prepared in vials (*) : 90 days at 2-8 °C.
Complete base media prepared in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without glycerol) :
500 g bottle BK165HA

Complete pre-poured media in Petri plates (Ø 55 mm) :
20 plates BM14508
120 plates BM19608

11 BIBLIOGRAPHY

Lowbury, E.J.L., and Collins, A.G., 1955. The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa*., J. Clin. Pathol., 8: 47.

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

Goto, S., and Enomoto S. 1970. Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of *Pseudomonas aeruginosa*.

Arrêté du 19 juin 2000 (JO du 20 juillet 2000) modifiant l'arrêté du 14 octobre 1937 modifié relatif au contrôle des sources d'eaux minérales.

NF EN ISO 16266. Août 2008. Qualité de l'eau. Détection et dénombrement de *Pseudomonas aeruginosa*. Méthode par filtration sur membrane.

NF T90-421. Aout 2006. Qualité de l'eau. Examens bactériologiques des eaux de piscine.

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.

Document code : CN_ENv8
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Updated : 07-2017
Origin of revision : Addition of the packaging BM19608

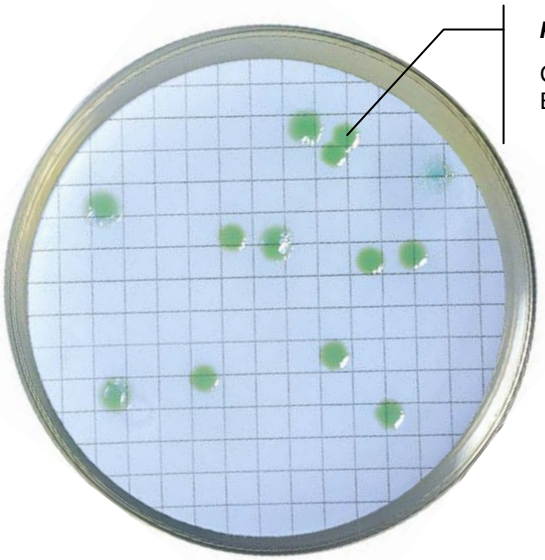
ANNEX 1 : PHOTO SUPPORT

CN Agar for *Pseudomonas*

Detection and enumeration of *Pseudomonas aeruginosa*.

Results :

Growth obtained after 44 hours of incubation at 36 °C (membrane filtration).



Pseudomonas aeruginosa

Characteristic colonies :
Blue-green colony.