

**Also known as**

CN Pseudomonas Agar; CN Medium; Cetrimide-Nalidixic Acid Medium

**Specification**

Selective solid medium used for the detection of *Pseudomonas aeruginosa* according to the EN 12780-2002 and ISO 16266 Standard.

**Formula \* in g/L**

Gelatin peptone.....	16.00
Casein peptone.....	10.00
Potassium sulfate.....	10.00
Magnesium chloride.....	1.40
Cetiltrimethyl-ammonium bromide.....	0.20
Agar.....	15.00

Final pH 7,1 ±0,2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

**Directions**

Add 52.6 g of powder to 1 L of distilled water with 10 mL of glycerol. Heat until completely dissolved. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 min. Cool to 45-50°C and to each 500 mL of medium add a vial of the Nalidixic Acid Selective Supplement (Art. No. DSHB3161). Mix well and pour into Petri dishes.

Do not allow the medium to remain in the molten state for more than 4 hours. Do not re-melt. The finished plates can be used without losing efficacy. For up to one month if they are refrigerated and kept in the dark.

**Description**

The CN Selective Medium for *Pseudomonas* was progressively developed from the basic medium of King, Ward and Raney for the enhanced production of pigments. Browne and Lowbury added cetrimide as a selective agent and Goto and Enomoto improved the selectivity by adding nalidixic acid. The presence of both inhibitors eliminates the contaminating microbiota from heavily polluted specimens and was adopted by ISO Standard for the detection of *P. aeruginosa* by membrane filtration of water.

**Necessary supplements**

Nalidixic Acid Selective Supplement (Art. No. DSHB3161)

Vial Contents:

Necessary amount for 500 mL of complete medium.

Nalidixic acid, sodium salt 7,5 mg

Distilled water (Solvent)

**Technique**

A volume of the sample is passed through a filter membrane of 0,45 µm pore and the membrane is then placed on the surface of the CN medium. The plates are incubated à 36 ± 2°C for a period of 44 ± 4 hours with a partial examination à 22 ± 2 hours.

All colonies producing a green or blue (pyocyanin) pigmentation in this period may be considered *Pseudomonas aeruginosa* and do not require further conformational testing.

All colonies that produce fluorescence under the Wood's light (without pyocyanin production) are considered presumptive *P. aeruginosa* but must be confirmed on Acetamide Medium.

All colonies producing a brown-reddish pigment and have no fluorescence or pyocyanine are also considered presumptive *P.aeruginosa* and must be confirmed by the oxidase test and by typical growth on Acetamide Medium and King B Agar (F Agar).

**Quality control**

**Incubation temperature:** 36°C ±2,0

**Incubation time:** 44±4h

**Inoculum:** Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity)/ 10<sup>4</sup>-10<sup>6</sup> CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018. MF methods.

Microorganism	Growth	Remarks
<i>Escherichia coli</i> ATCC® 8739	Inhibited	-
<i>Enterococcus faecalis</i> ATCC® 29212	Inhibited	-
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Productivity > 0.50	-
<i>Pseudomonas aeruginosa</i> ATCC® 10145	Productivity > 0.50	-
<i>Pseudomonas aeruginosa</i> ATCC® 9027	Productivity > 0.50	-

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**References**

- BROWN, V.L. & E.J.L. LOWBURY (1965) Use of an improved Cetrimide Agar Medium and of culture methods for *P. aeruginosa*. J., Clin. Pathol. 18:752.
- GOTO S. & S. ENOMOTO (1970) Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of *P. aeruginosa*. Jpn. J. Microbiol. 14:65.
- ISO 16266 Standard (2006) Water Quality. - Detection and enumeration of *Pseudomonas aeruginosa*. - Method by membrane filtration.
- KING, E.O., M.K. WARD & E.E. RANEY (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301.
- ROBIN, T. & J.M. JANDA (1984) Enhanced recovery of *P. aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect Dis. 2:207.
- SCHWEIZERISCHE LEBENMITTELSBUCH (2005) Kap. 56 Mikrobiologie. Bundesamt für Gesundheit. Direktionsbereich Verbraucherschutz. Bern.

**Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).