Pseudomonas CN Agar Base ISO

Cat. 1153

For the identification and enumeration of Pseudomonas aeruginosa by membrane filtration

Practical information

Aplications	Categories
Selective enumeration	Pseudomonas aeruginosa
Detection	Pseudomonas aeruginosa

Industry: Water

Regulations: ISO 11133 / ISO 16266

Principles and uses

Pseudomonas CN Agar Base is used for the identification of Pseudomonas aeruginosa by membrane filtration technique, based on the detection of pyocyanin production. It is a modification of Pseudomonas P Agar (King A Medium - Cat. 1531). This medium is recommended by ISO 16266.

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water should be checked to be free of Pseudomonas aeruginosa at the time of commercialization. This microorganism can also be found in swimming pool water.

Peptone and casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Cetrimide is added as a selective agent, and nalidixic acid to suppress contaminants of cetrimide media such as Klebsiella, Proteus and Providencia spp. Potassium sulfate and magnesium chloride provide cations to activate pyocyanin production and enhance pigment production. Bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	13	Cetrimide	0,2
Gelatin peptone	16	Magnesium chloride anhydrous	1,4
Nalidixic acid	0,015	Casein hydrolysate	10
Anhydrous potassium sulfate	10		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 50,6 grams of the medium in one liter of distilled water. Add 10 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118 ºC for 15 minutes. Cool to 45-50 ºC, mix well and dispense into plates to obtain an agar layer of at least 5 mm thick. Do not remelt the medium.

Instructions for use

According to ISO 16266 for the detection and enumeration of Pseudomonas aeruginosa:
- Filter a certain volume of water sample through a filter membrane and place the membrane on a Pseudomonas CN Agar Base plate (Cat. 1153).
- Incubate at a temperature of 36±2 ºC for 44±4 h.
- Count the colonies that have a green/blue pigmentation (pyocyanin) as confirmed P. aeruginosa.
- Examine the membrane under UV light.
- All colonies that are fluorescence (+) and reddish-brown colonies should be confirmed.
- Spread all the colonies that should be confirmed on Nutrient Agar plates (Cat. 1156) to obtain pure cultures. Incubate at 36±2 ºC for 22±2 h
- Perform oxidase assay to the reddish-brown colonies.
- Streak the oxidase (+) colonies on King B Medium (Cat. 1532) to check the fluorescence production. Incubate at 36±2 ºC for up to 5 days. Normally 24 hours are enough.
- Inoculate all the fluorescence (+) colonies, both in CN agar and in King B Medium, in the Acetamide Broth (Cat. 1155 o Cat.2017) medium and add one or two drops of Nessler reagent to check the ammonia production. Incubate at 36±2 ºC for 22±2 h.
- The colonies that produce pyocyanin in CN agar, the colonies fluorescence (+) in CN agar and ammonia (+) in Acetamide broth, and the reddish brown colonies in CN agar, oxidase (+), fluorescence (+) in King B Agar and ammonia (+) in Acetamide Broth, are counted as confirmed P. aeruginosa.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber slightly opalescent	7,1±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (36±2 ºC / 22±2 h)
Inoculation conditions: Productivity quantitative (100±20.Min.50 CFU), Selectivity (10^4-10^6 CFU).
Reference media: TSA.

Microorganisms	Specification	Characteristic reaction
Enterococcus faecalis ATCC 19433	Total inhibition (0)	
Escherichia coli ATCC 25922	Total inhibition (0)	
Pseudomonas aeruginosa ATCC 27853	Good growth >50%	Blue-green colonies with fluorescence under UV light (360 ± 20 nm)
Pseudomonas aeruginosa ATCC 9027	Good growth >50%	Blue-green colonies with fluorescence under UV light (360 ± 20 nm)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

UNE-EN 12780: 2002, Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration.
EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration