Acetamide Broth ISO

Cat. 2017

For confirmation of Pseudomonas aeruginosa by membrane filtration

Practical information

Aplications	Categories
Confirmation	Pseudomonas

Industry: Water

Regulations: / ISO 16266

Principles and uses

Acetamide Broth contains acetamide which is the sole source of carbon. It is used for the confirmation and identification of Pseudomonas aeruginosa, as specified by the ISO 16266. It uses the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide. The deamination of the acetamide produces ammonia which increases the pH of the medium, acetamide deamination is accomplished by P.aeruginosa, P. acidovorans, Group III (Achromobacter xylosoxidans), and Alcaligenes odorans.

Acetamide is the single carbon source. The Potassium salt has a high buffering capacity and Sodium chloride supplies essential electrolytes for transport and osmotic balance. Magnesium sulfate, sodium molybdate and iron sulfate allow the selective growth of Pseudomonas in the medium.

It is prepared according to 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 are Pseudomonas aeruginosa free at the time of their commercialization, This mircroorganism can also be found in swimming pool water.

Formula in g/L

Acetamide	2	Ferrous sulfate	0,0005
Magnesium sulfate	0,2	Monopotassium phosphate	1
Sodium chloride	0,2	Sodium molybdate	0,005

Preparation

Suspend 3,4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Distribute into tubes in 5 ml aliquots, close and sterilize in autoclave at 121 °C for 15 minutes.

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 °C 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. 1154) 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	Colorless	7,0 ± 0,5

Microbiological test

Incubation conditions: (36±2 ºC / 22±2 h)

Microrganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Good growth	Ammonium production
Pseudomonas aeruginosa ATCC 27853	Good growth	Ammonium production
Pseudomonas aeruginosa ATCC 9027	Good growth	Ammonium production

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration