Specification

Liquid test medium use for the confirmation of *Pseudomonas aeruginosa* in water according to the ISO 16266:2006 standard.

Formula * in g/L

Acetamide	2.0000
Magnesium sulfate	0.2000
Sodium chloride	0.2000
Ferrous sulfate	0.0005
Mono-potassium phosphate	1.0000
Sodium molybdate	0.0050

Final pH 7,0 ±0,5 at 25 ºC

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

Directions

Dissolve 3.4 g of powder in 1 L of distilled water. Heat only if necessary. Distribute in screw-capped tubes and sterilize in the autoclave at 121ºC for 15 minutes. Prepared medium may be opalescent and with precipitate. To obtain a clear transparent medium without precipitation, avoid heating and sterilize the medium by filtration. The sterilized medium (with or without precipitates) remains active for 3 months if it is stored in the dark in a cool place.

Description

This nutrient solution uses acetamide as the sole carbon and nitrogen source, and therefore it only allows the growth of those microorganisms that are able to use acetamide. In water and in almost all food stuffs, these microorganisms are the non fermenting Gram negative bacillis, *Pseudomonas aeruginosa* is the only organism that can liberate ammonia by deamination of acetamide.
Some authors suggest the use of this nutrient solution as an enrichment medium prior to the use of isolation medium, will reduce false positives from heavily polluted samples. *Comamonas acidovorans*, *Achromobacter xylosoxidans* and *Alcaligenes faecalis (odorans)* can also deaminate acetamide, but can not growth on the plating medium that is selective for *Pseudomonas*.

Technique

To confirm potencial Pseudomonas aeruginosa colonies on the Cetrimide Agar, they must first be cultured on a non-selective medium to obtain pure cultures from which perform the confirmation tests.
Acetamide Medium is inoculated with a couple of colonies from the pure culture and is incubated at 36 ± 2ºC for 22 ± 2 hours. Add 2 drops of Nessler's Reagent and examine the tubes for the production of ammonia, characterized by the production of a colour varying from yellow to brick red depending upon concentration of ammonia present.

Quality control

Incubation temperature: 36°C ±2,0 **Incubation time:** 22h ± 2
Inoculum: ≥ 10^3 CFU (specificity) according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020.

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 27853	Good	Green pigment. Acet (+)
Pseudomonas aeruginosa ATCC® 10145	Good	Green pigment. Acet (+)
Pseudomonas aeruginosa ATCC® 9027	Good	Green pigment (48 h). Acet (+)
Escherichia coli ATCC® 25922	Inhibited	-

References

· DIN Standard 3841. Deutsche Einheitsverfahren zür Wasser, Abwasser und Schlammuntersuchung Mikrobiologische Verfahren: Nachweiss von Pseudomonas aeruginosa (K8).
· ISO 16266 Standard (2006) Water Quality - Detection and enumeration of Pseudomonas aeruginosa - Method by membrane filtration.
· KELLY, N.M., C.T. KEANZ (1983) Acetamide Broth for Isolation of Pseudomonas aeruginosa from patients with cystic fibrosis. J. Clin. Microbiol. 17.

Storage

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