Specification

Solid medium for the enumeration of heterotrophic microorganisms in treated waters, according to Pharmacopoeial Harmonized Methods.

Formula * in g/L

Proteose peptone	0.500	Dipotassium phosphate	0.300
Casein peptone	0.500	Magnesium sulphate (anhydrous)	0.024
Yeast extract	0.500	Agar	15.000
D(+)-Glucose	0.500		
Starch	0.500		
Sodium pyruvate	0.300		

Final pH 7.2 ±0.2 at 25 °C

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

Directions

Suspend 18,1 g of powder in 1 L of distilled water and bring to the boil constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description

R2A Agar was proposed in 1979 by Reasoner and Geldenreich and a few years later accepted by the APHA as an alternative medium for the enumeration of stressed cells in treated potable water.
The use of nutrient rich media like PCA or TSA allows the growth of most microbes, but does not permit the recuperation of stressed or chlorine resistant organisms. Using a medium like R2A with low nutrients in combination with a lower temperature and longer incubation time it is possible to induce the resuscitation of these damaged cells.
In R2A Agar the source of nitrogen is the peptone and Yeast Extract supplies the vitamins and growth factors. The source of carbon is dextrose and magnesium sulfate and potassium phosphate maintain the osmotic pressure. The starch is a detoxifier and sodium pyruvate increases the recuperation of stressed cells. The agar acts as gelling agent.

Technique

The water sample must be processed as quickly as possible. If it is not possible to process within the first 6 hours, the sample must be refrigerated, but not for more than 30 hours.
R2A Agar can be used for pour plates, streak plates or filtration. The pour plate method can affect the recovery capacity of the medium because due to thermal shock when mixing molten agar with the sample. Incubating à 35 °C, an incubation period of 3-5 days is recommended. In most circumstances an incubation temperature of 20-28 °C for 5-7 days is more effective. Plates must be protected against dehydration.

Quality control

Incubation temperature: 30-35 °C / 20-28 ºC **Incubation time:** 48-72 h / ≤ 5 days

Inoculum: Practicalrange 50-100 CFU (productivity), according to Ph. Eur. and ISO 11133:2014/Amd 1:2018. Membrane filter method x 2 Temperatures.

Microorganism	Growth	Remarks
Bacillus subtilis ATCC® 6633	Productivity > 0.70	-
Staphylococcus aureus ATCC® 6538	Productivity > 0.70	-
Pseudomonas aeruginosa ATCC® 9027	Productivity > 0.70	-
Escherichia coli ATCC® 25922	Productivity > 0.70	-
Salmonella abony NCTC® 6017	Productivity > 0.70	-
Candida albicans ATCC® 10231	Productivity > 0.70	-
Aspergillus brasiliensis ATCC® 16404	Productivity > 0.70	-

References

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· EUROPEAN PHARMACOPOEIA. 10th ed. Suppl 6.3 (2020) General Monographs. Water for injections. (pg. 4339) EDQM. Council of Europe. Strasbourg.
· GREENBERG, A.E., R.R. TRUSSELL and L.S. CLESCERI (1985). Standard Methods for the Examination of Water and Wastewater. 16th ed. APHA-AWWA-WPCF. Washington D.C. USA.
· REASONER, D.J. and E.E. GELDREICH (1979) A new Medium for the enumeration and subculture of bacteria from potable water. Abstracts of Annual Meeting. ASM 79th Meeting. Paper #N7.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· Van SOETSBERGER, A.A. and C.H. LEE (1969) Pour plates or streak plates?. Appl. Microbiol. 18:1092 -1094.

Storage

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