Condalab

Reference: 4711 **Technical Data Sheet**

Product: R2A AGAR (EUROPEAN PHARMA)

Specification

Solid medium for the enumeration of heterotrophic microorganisms in treated waters according to Pharmacopoeial Method.

Presentation

	Packaging Details	Shelf Life	Storage
30 Prepared plates 55 mm Plates for filtration purposes with: 9 ± 1 ml	1 box containing: 5 plastic bags with 6 plates of 55 mm/ bag.	6 months	2-25°C

Composition

Composition (g/l):

Proteose peptone	0.500
Casein hydrolysate (Tryptone)	0.500
Yeast extract	0.500
D(+)-Glucose	0.500
Starch	0.500
Sodium pyruvate	0.300
Dipotassium hydrogen phosphate	0.300
Magnessium suphate (anhydrous)	0.024
Agar	15.000

Description /Technique

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 culture medium has also been adopted by the European Pharmacopoeia for the control of purified 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.

Collect, dilute and prepare samples and volumes to be filtered as required according to specifications, directives, official standard regulations and/or expected results.

Filter the sample through a 0.45 mm pore membrane and apply it onto the surface of the agar.

he incubating at 35°C, an incubation period of 3-5 days is recommended. In most circumstances an incubation temperature of 20 -25°C for 5-7 days is more effective. Plates must be protected agains dehydration.

After incubation, enumerate all the colonies that have appeared onto the surface of the membrane.

Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by the inverse dilution factor. Report results as Colony Forming Unit (CFU's) per ml along with incubation time and temperature.

Quality control

Physical/Chemical control

Color : Pale yellow pH: 7.2 ± 0.2 at 25°C

Microbiological control

Growth Promotion Test according to harmonized pharmacopoeial monographs and test methods & ISO 11133:2014
Membrane Filtration /Practical range 100±20 CFU; Min. 50 CFU (Productivity)./10^4-10^6 CFU for Selectivity.
Aerobic.Incubation at 30-35°C for 24-48h (bacteria) and 20-25°C for 3-5 days (moulds and yeast).

Microorganism	Growth
Ps. aeruginosa ATCC® 9027, WDCM 00026	Good (≥70 %)
Bacillus subtilis ATCC® 6633, WDCM 00003	Good (≥70 %)
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥70 %)
Aspergillus brasiliensis ATCC® 16404, WDCM 00053	Good (≥70 %)
Candida albicans ATCC® 10231, WDCM 00054	Good (≥70 %)
Staphylococcus aureus ATCC® 6538, WDCM 00032	Good (≥70 %)

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH
Check at 7 days after incubation in same conditions

Bibliography

· ATLAS, R.M. (1995) Handbook of Media for Environmental Microbiology. CRC Press. Boca Raton. Fla. USA.
· CLESCERI, L.S., A.E. GREENBERG and A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA Washington D.C. USA.
· EATON, A.D., A.E. GREENBERG and L.S. CLESCERI (1995). Standard Methods for the Examination of Water and Wastewater. 19th ed. APHA Washington D.C. USA.
· EUROPEAN PHARMACOPOEIA. 6th ed. Suppl 6.3 (2009) 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.
· Van SOETSBERGER, A.A. and C.H. LEE (1969) Pour plates or streak plates?. Appl. Microbiol. 18:1092 -1094.