

## Specification

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

## Presentation

	Packaging Details	Shelf Life	Storage
20 Plates /Ird. 90 mm - Double wrapping with: 21 ± 2 ml	1 box with 2 cellophane bags (double wrapping) with 10 plates/bag. Side labeling. Every pack exhibits a irradiation indicator stacked on the side of the bag.(8 -14kGy).	3,5 months	2-14 °C

## Composition

Composition (g/l):	
Proteose Peptone.....	0.500
Casein Peptone.....	0.500
Yeast extract.....	0.500
Glucose.....	0.500
Soluble starch.....	0.500
Sodium pyruvate.....	0.300
DiPotassium phosphate.....	0.300
Magnesium sulfate .....	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.

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. The 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 against dehydration.

**Attention:** Petri plates are used for monitoring the microbiological contamination of surface and air inside cleanrooms, isolators, RABS, food industries and hospitals. The double/triple irradiated wrapping ensures that the package itself doesn't contaminate the environment as the first wrapper is removed just before entering the clean area.

**Quality control****Physical/Chemical control**

Color : Pale yellow

pH: 7.2 ± 0.2 at 25°C

**Microbiological control**

Membrane Filtration; Practical range 10-100 CFU (productivity) according Eur. Pharm.

Aerobiosis. Incubation at 32,5°C ±2,5. Reading at 24-72 h for bacteria and 5-7 days for yeasts and moulds

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

*Ps. aeruginosa* y *E. coli* double incubation temp. 30-35 °C / 20-25 °C

Microbiological control according to ISO 11133:2014/A1:2018; A2:2020.

**Microorganism***Escherichia coli* ATCC® 8739, WDCM 00012*Staphylococcus aureus* ATCC® 6538, WDCM 00032*Bacillus subtilis* ATCC® 6633, WDCM 00003*Candida albicans* ATCC® 10231, WDCM 00054*Ps. aeruginosa* ATCC® 9027, WDCM 00026*Aspergillus brasiliensis* ATCC® 16404, WDCM 00053*E. coli* ATCC® 8739, WDCM 00012 (20-25°C)*Ps. aeruginosa* ATCC® 9027, WDCM 00026 (20-25°C)**Growth**

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

**Sterility Control**

Check at 7 days after incubation in same conditions.

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

**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.

**Storage**

Storage conditions: 2-14°C

Alternatively the plates may also be stored at the range of 2 - 25°C, with a proper performance of the medium, but some precautions must be taken into account:

-In the range of 2 - 8 °C avoid direct contact with surfaces that can freeze product.

-In the range of 15 - 25 °C, dehydration control must be taking in account.