Condalab

Reference: 0985 **Technical Data Sheet**

Product: # LEGIONELLA AGAR [CYE / GVPC] ISO 11731-2

Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* from water according to the ISO Standards 11731.

Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 22 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14°C

Composition

Composition (g/l):

Activate charcoal	2.000
Yeast extract	10.000
Aces buffer	10.000
Potassium hydroxide	2.800
Alfa-ketoglutarate	1.000
Cysteine HCl	0.400
Ferric pyrophosphate	0.250
Glycine (ammonia free)	3.000
Vancomycin	0.001
Polymixin B	80.000 UI
Cycloheximide	0.0800
Agar	15.000

Description /Technique

Description:

The actual formulation of this medium is according to the ISO Standards 11731: 2017, but BCYE Agar is based in a modification of a previously described media. In 1979 Feeley and collaborators described Charcoal Yeast Extract (CYE) Agar as a modification of the F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in a better recovery of *Legionella pneumophila*. Pasculle, in 1980, reported that CYE Agar could be improved by buffering the medium with ACES buffer and a year later Edelstein increased the sensitivity of the medium by adding a-ketoglutarate which is the present formulation (BCYE Agar).

The medium consist of a Medium base supplemented with growth factors (BCYE Agar) and the Selective Medium supplemented with inhibitors of undesirable accompanying flora. The yeast Extract supplies the basic nutrients as the medium contains no fermentable carbohydrates. L-Cysteine, Ferric pyrophosphate and a-ketoglutarate are incorporate to satisfy the specific nutritional requirements of *Legionella* species.

The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO_2 and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is raised by the addition on Vancomycin or Sodium cefazolin they acts against gram-positive bacteria, Polymyxin B that inhibits gram-negative bacteria, anisomycin that has a broad spectrum of activity and Cycloheximide or Natamycin that as antifungal agents inhibits the yeast growth.

Technique:

Refer to the ISO Standards 11731:2017 or other standard procedures to obtain isolated colonies from specimens and samples. Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate at 36 ± 2°C for up to 2, 3, 5 -10 days. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Top up this tray with fresh water (if necessary) each time the plates are examined. Incubation in an atmosphere of air with 2,5% (volume fraction) CO_2 may be beneficial for the growth of some *Legionella*, but it is not essential.

Examine the plates with a plate microscope on at least three occasions at intervals of 2,3 to 5 days during the 10-day incubation period, as *Legionella* grow slowly an can be masked by the growth of other organisms. Record the number of each type of colony present.

Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. They are smooth with a smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species autofluoresce brilliant white, but others are red and *L. pneumophila* appear dull green often tinged with yellow. All presumptive colonies must be confirmed by cultural, biochemical, serological or genetic methods.

Note: If the medium is used with the membrane filter method, the colour and growth of the colonies may be effected. It is advisable to perform validation of the membrane filter used, by the thechnical.

Quality control

Physical/Chemical control

Color : Black pH: 6.8 ± 0.2 at 25°C

Microbiological control

Spiral Spreading /MF Methods; Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity).
Microbiological control according to ISO 11133:2014/ Adm 1:2018.

Aerobiosis. Incubation at 36 ± 2 °C. Reading 3 - 5 days, up to 10 days.

Microorganism	Growth
L. anisa ATCC® 35292, WDCM 00106 (by MF)	Good (≥ 70%) grey-blue colonies
Legionella anisa ATCC® 35292, WDCM 00106	Good (≥ 70%) grey-blue colonies
L. pneumophila ATCC® 33152, WDCM 00107 (by MF)	Good (≥ 70%) grey-blue colonies
Legionella pneumophila ATCC® 33152, WDCM 00107	Good (≥ 70%) grey-blue colonies
Escherichia coli ATCC® 25922, WDCM 00013	Inhibition (Partial to complet)
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
Membrane filter NALGDS0205-6045, batch used:	F7KA35617
the reference medium is GVPC validated.	

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. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. BocaRaton. Fla. USA.

· CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard methods for the examination of water and wastewater. 9-106. 20th edition. APHA-AWWA-WEF. Washington DF, USA.

· EDELSTEIN, P.H., (1981) Improved semiselective medium for the isolation of *Legionella pneumoniae* from contaminated clinical and environmental specimens. J. Clin Microbiol. 14(3):298.

· FEELEY, J.C., R.J. GIBSON, G.W. GORMAN, N.C. LANGFORD, J.K. RASHEED, C.D. MACKEL, & W.B. BAINE (1979) Charcoal-Yeast Extract Agar: Primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol. 10(4) 437.

· ISO 11731 Standard (2017) Water Quality - Enumeration of *Legionella*.

. ISO 11133:2014/ Adm 1:2018/ Adm1 :2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

· MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria.

· PASCULLE, A.W., J.C. FEELEY, R.J. GIBSON, L.G. CORDES, R.L. MYEROWITZ, C.M. PATTON, G.W. GORMAN, C.L. CARMACK, J.W. EZZELL & J.N. DOWLING (1980) Pittsburgh pneumonia agent: Direct isolation from human lung tissue. J. Infect. Dis., 141:727.

. UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.

· WARD, K.W. (1995) Processing and interpretation of specimens for *Legionella spp*. In "Clinical Microbiology Procedures Handbook" Chap. 12.1 edited b H.D. Isenberg. ASM Press. Washington DC, USA.