Instructions For Use
ENGLISH

Legionella Agar (GVPC) (RT)

Selective isolation medium for detection and enumeration of *Legionella* spp in water, according to ISO 11731.

DESCRIPTION

Legionella Agar (GVPC) is a selective medium used for primary isolation of *Legionella* spp from water samples.

This medium conforms to the recommendations of ISO 11731 and ISO 11731-2 for examination of all kinds of environmental samples including potable, industrial and natural waters and associated materials as well.

TYPICAL FORMULA	(g/l)
Yeast Extract	10.0
Charcoal Activated	2.0
ACES Buffer	10.0
Potassium Hydroxyde	2.8
α-Ketoglutarate	1.0
Ferric Pyrophosphate	0.25
L-Cysteine	0.4
Agar	13.0
Glycine	3.0
Vancomycin	0.001
Polymyxin B	80000 IU
Cycloheximide	0.08

Final pH 6.9 ± 0.2 at 25°C

METHOD PRINCIPLE

Yeast extract provides amino acids, nitrogen, carbon, vitamins and minerals. Activated charcoal decomposes hydrogen peroxide, a metabolic product toxic to *Legionella* spp, and may also collect carbon dioxide and modify surface tension. ACES buffer (N-2- acetamido-2-aminoethane sulfonic acid) and potassium hydroxide maintain the proper pH for optimal growth. Alpha-ketoglutarate, cysteine and ferric pirophosphate are incorporated to satisfy the specific nutritional requirements of Legionella species. Agar is the solidifying agent. Glycine, vancomycin and polymyxin B inhibit or suppress most non-target bacterial species, both Gram-positive and Gram-negative, including common contaminants such as enterococci, coliform, and *Pseudomonas* spp, while cycloheximide suppresses the growth of yeasts and moulds.

TEST PROCEDURE

According to ISO 11731, perform direct analysis if the number of *Legionella* is expected to exceed 10^5 per liter. Otherwise, samples need to be concentrated by membrane filtration or by centrifugation prior to culture. To reduce the growth of unwanted bacteria, two portions of sample, concentrated or not, are treated either with heat or acid. A third portion is left without any further treatment.

For liquid samples, inoculate by spreading 0.1 to 0.5 ml of the test over the agar surface, or filter 10 to 1000 ml of water sample before placing the membrane onto the agar.

Incubate at 36 ± 2°C for up to 10 days in humidified atmosphere (air with 2.5% CO_2 can be beneficial for the growth of some *Legionella* but is not essential).

Regarding sample transport and storage, analysis should begin as soon as possible after the sample has been taken, preferably on the same day. Samples should be protected from heat and sunlight, kept between 6-20°C and transported to the laboratory within 24 hours of collection.

INTERPRETING RESULTS

Examine for growth and fluorescence under long-wave UV light on at least two occasions during the incubation period starting from the third day.

Colonies of *Legionella* on black media or black membrane are often white-grey-blue-purple in colour, but can be brown, pink, lime-green or deep-red. They are smooth with an entire edge and exhibit a characteristic ground-glass appearance. Under UV light colonies usually exhibit brilliant blue-white fluorescence. Colonies of *L. pneumophila* appear dull-green often tinged with yellow.

Select colonies characteristic of *Legionella* on each plate for subculture onto plates of Legionella BCYE Agar (ref. 10051) and Legionella BCYE Agar w/o cysteine (ref. 10412). Incubate at 36 ± 2°C for at least 2 days in humidified atmosphere. Regard as Legionella those colonies which grow on Legionella BCYE Agar but fail to grow on Legionella BCYE Agar w/o cysteine. Confirm by serological tests.

APPEARANCE

Opaque, black.

STORAGE

Store at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

6 months.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Inoculum for selectivity: 10^4-10^6 CFU.
Incubation conditions: 36 ± 2°C for up to 10 days in humidified atmosphere.

QC Table.

Microorganism		Growth
Legionella pneumophila	WDCM 00107	Good
Legionella anisa	WDCM 00106	Good
Enterococcus faecalis	WDCM 00087	Inhibited
Pseudomonas aeruginosa	WDCM 00025	Inhibited
Escherichia coli	WDCM 00013	Inhibited

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

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2. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
3. ISO 11731-2:2004. Water Quality – Detection and Enumeration of Legionella. – Direct membrane filtration method for waters with low bacterial count.
4. ISO 11731:1998. Water Quality – Detection and Enumeration of Legionella.
5. Clesceri L.S., A.E. Greenberg and A.D. Eaton (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association (APHA), Washington, D.C.
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8. McDade, J.E., Shepard, C.C., Faser, D.W. et al. (1977) Legionnaires' disease : isolation of a bacterium and demonstration of its role in other respiratory disease. New England Journal of Medicine, 297:1197-1203.

Product	Format	Packaging	Ref.
Legionella Agar (GVPC) (RT)	90 mm Plate	20 plates	10998

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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