

INSTRUCTIONS FOR USE

LEGIONELLA AGAR W/O CYSTEINE

Ready-to-use plates

1 - INTENDED USE

In vitro diagnostic device. Medium without L-cysteine for the confirmation of *Legionella* colonies isolated from clinical specimens and water samples.

2 - COMPOSITION - TYPICAL FORMULA *

Activated charcoal	2.0 g
Yeast extract	10.0 g
Agar	13.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
α -ketoglutarate	1.0 g
Distilled water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionellae are mesophilic, motile, a-saccharolytic, obligate aerobic, nutritionally fastidious, Gram-negative, non-spore-forming gammaproteobacteria.¹ *Legionella pneumophila*, the most widely studied species, displays pleomorphism, demonstrating coccoid, bacillary and/or long filamentous forms that are influenced by temperature, available nutrients or metabolites, growth environment and medium type.² *Legionella* species share growth dependence for L-cysteine and growth enhancement by iron.¹ Legionellae grow on several types of complex artificial media, however, the most successful medium is buffered charcoal yeast extract (BCYE) agar containing ferric pyrophosphate, α -ketoglutarate and L-cysteine.²

Colonies isolated on Legionella selective and non-selective media are identified by colonial morphology, Gram stain and by the requirement of L-cysteine for growth.

Legionella spp. will grow on Legionella Agar supplemented with L-cysteine. Suspect *Legionella* species will not grow on the same medium from which L-cysteine has been omitted. Growth on both plates indicates that the organism is not *Legionella*.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	black, homogeneously opaque
Final pH at 20-25°C	6.9 \pm 0.1

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Legionella Agar w/o Cysteine	Ready-to-use plates	549943	2 x 10 plates \varnothing 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the complete identification of the colonies.

7 - SPECIMENS

Legionella Agar w/o Cysteine must be inoculated with colonies cultivated on selective or non-selective BCYE Agar.

8 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Presumptive colonies are confirmed as *Legionella* by subculture to demonstrate their growth requirement for L-cysteine.

When there is only one colony type, pick three presumptive colonies; if more morphological different types of presumptive colonies of *Legionella* are growing on the plate, take at least one colony from each type.³

Subculture onto a plate of Legionella Agar (BCYE) (REF 549945) and a plate of Legionella Agar w/o Cysteine.

Be careful not to carry over any culture medium with the colony and first inoculate a plate of Legionella Agar w/o Cysteine.

Incubate at 36 \pm 2°C for 2 to 5 days.³

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth on both inoculated plates. Regard as *Legionella* those colonies which grow on the plate of Legionella Agar (BCYE) but fail to grow on the plate of Legionella Agar w/o Cysteine.

Presumptive identification should be completed by Gram staining prepared from cysteine containing agar only: *Legionella* cells are Gram-negative poorly/faintly staining thin rods, which may be filamentous in older cultures.⁴

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.





CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>L.pneumophila</i> ATCC 33152	35-37°C / 44-48 h / A	no growth
<i>E.coli</i> ATCC 25922	35-37°C / 18-24 h / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Legionella Agar w/o Cysteine is tested for the specific performance characteristic.

Legionella Agar w/o Cysteine is tested with a target strain (*L. pneumophila* ATCC 33152) and a non-target strain (*E.coli* ATCC 25922). After incubation at 35-37°C for 44-48 hours *L. pneumophila* fails to growth, while *E.coli* show a good growth after 18-24 hours incubation.

12 - LIMITATIONS OF THE METHOD

- Some rare *Legionella* spp. (*L.oakridgensis*, *L.jordanis*, *L.nagasakiensis*, *L.spiritensis*) may adapt to cysteine-deficient media after serial passage and may grow slowly and slightly^{2,3,4} Accordingly, careful comparison needs to be made of the differences in growth between cysteine-supplemented and un-supplemented culture media.³
- The plates with characteristic growth and with colonies presumptively identified as *Legionella*, must undergo confirmation tests with biochemical, immunological, molecular or mass spectrometry techniques. If relevant, perform antimicrobial susceptibility testing.
- In clinical microbiology, the diagnosis of legionellosis must be based on an interdisciplinary approach that includes radiological results, cultural results, determination of urinary antigen. Legionella Agar w/o Cysteine is intended as an aid to the diagnosis of the infection: the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- When handling *Legionella* spp., it is important to avoid aerosol formation. Thoroughly clean and disinfect all work areas.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

- Edelstein PH, Luck C. *Legionella*. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015.
- Mercante JW, Winchell JM. Current and Emerging Legionella Diagnostics for Laboratory and Outbreak Investigations. Clin Microbiol Rev. 2015; 28:95-147
- ISO 11731:2017 Water quality — Enumeration of Legionella
- Public Health England. UK Standards for Microbiology Investigations. Identification of Legionella species. ID18, Issue no: 3, Issue date: 14.04.15

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD In vitro Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 6	Updated layout and content in compliance with IVDR 2017/746	2020/10
Revision 7	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

