Instructions for Use

REF - PP0870 **Legionella GVPC Agar**

Pre-Poured Media

1. Intended Use

PP0870 Legionella GVPC Agar is a selective medium for the isolation of *Legionella* spp. used primarily in water and environmental laboratories.

2. Composition*

Ingredient	g/L
Yeast extract	10.0
Agar	14.0
Charcoal	2.0
α-ketoglutaric acid	1.0
ACES buffer	10.0
Potassium hydroxide	2.8
L-cysteine hydrochloride	0.4
Iron (III) pyrophosphate	0.25
Glycine	3.0
Vancomycin	0.001
Polymyxin B	80,000IU
Cycloheximide	0.08

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Legionella spp. are aerobic, motile, nutritionally fastidious, Gram-negative rods that require special growth factors in culture media to grow. Transmission occurs through aerosolization or aspiration of contaminated water. Over fifty species of *Legionella* have been identified, however *Legionella pneumophila* remains the most prevalent etiological agent of Legionnaires' disease.

PP0870 conforms to the recommendations of ISO 11731 [1] for examination of all kinds of environmental samples including potable, industrial and natural waters, and associated materials.

4. Principle

Since *Legionella* species are fastidious organisms, the medium contains yeast extract as a source of nitrogen, carbon and vitamins. Charcoal is also included to neutralise growth-inhibiting substances and agar is the solidifying agent [2]. ACES buffer/potassium hydroxide is added to maintain the optimal pH of 6.9 for growth of *Legionella* species. The medium includes iron (III) pyrophosphate as a source of iron, L-cysteine as an essential amino acid for the growth of *Legionella* species and α-ketoglutaric acid, which acts as a growth stimulant [3]. The medium is made selective by the addition of glycine, vancomycin and polymyxin B to inhibit most Gram-positive and Gram-negative organisms and cycloheximide is also included to inhibit yeasts and fungi.

5. Physical Characteristics

	Medium
Appearance and Colour	Black firm gel
pH	6.9 ± 0.2

6. Materials Provided

PP0870 Legionella GVPC Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0870-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP0870 Legionella GVPC Agar is suitable for the testing of the following specimens:

- Water Specimens: Potable water, spa waters, cooling tower water and environmental and domestic water samples [4].

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Allow media to come to room temperature.

Water Specimens [1]

According to ISO 11731:2017, the method required for enumerating *Legionella* spp. is determined by the assumed sample content. PP0870 Legionella GVPC Agar is recommended when processing samples that are presumed to have higher numbers of interfering microorganisms in combination with lower concentrations of *Legionella* species.

- Process the water/environmental sample as required by the local guidelines.
- Inoculate samples onto the selective medium PP0870 Legionella GVPC Agar, and onto non-selective media Legionella BCYE Agar (E&O PP0200) if required.
- Incubate the plates 36 ± 2°C in 5% CO_2 and high humidity for up to 7 days (further incubation up to 10 days may be required to present growth of wild *Legionella* strains).

Colonies suspected of being *Legionella* spp. are subcultured to Legionella BCYE Agar and Legionella BCYE Cysteine Free Agar (E&O PP0201).

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
L. pneumophila (NCTC 11192)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Growth: White/grey colonies
L. anisa (NCTC 11974)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Growth: White/grey colonies
E. coli (NCTC 12241)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Inhibited
S. aureus (NCTC 12981)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Inhibited
S. cerevisiae (NCPF 3178)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Inhibited
P. aeruginosa (NCTC 12903)	37 ± 1°C in 5% CO_2 (with high humidity) for 2-5 days	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify PP0870 Legionella GVPC Agar performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4-10^7 cfu for the non-target organisms then incubated at 37 ± 1°C for 2-5 days. All samples showed expected recovery and morphology of the required test organisms: *Legionella pneumophila* (NCTC 11192) and *Legionella anisa* (NCTC 11974), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Staphylococcus aureus* (NCTC 12981), *Saccharomyces cerevisiae* (NCPF 3178) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that PP0870 Legionella GVPC Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations

- The glycine contained in the medium may inhibit some of non-pneumophila strains.
- Due to natural variation, some strains may grow poorly on this medium.
- Colonies of *Legionella* spp. grown on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non-selective and selective media is strongly recommended.
- The plates with characteristic growth and with colonies presumptively identified as *Legionella*, must undergo confirmation tests with biochemical, immunological, molecular, or mass spectrometry techniques. These tests should be performed from a non-selective agar.
- Thermophilic spore-bearing organisms may mimic *Legionella* colonies at 35°C incubation. These organisms can be detected by incubating parallel plates at 35°C and 55°C when the thermophilic organisms grow, but *Legionella* species will not.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0870 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date or if cracks in the agar medium are present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. International Organization for Standardization (2017) 11731:2017 Water quality - Enumeration of *Legionella*. Geneva, ISO.
2. Feeley, J., Gibson, R., Gorman, G., Langford, N., Rasheed, J., Mackel, D., and Baine, W. (1979) Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. Journal Of Clinical Microbiology, 10(4), pp.437-441.
3. Fields, B., Benson, R., and Besser, R. (2002) *Legionella* and Legionnaires' Disease: 25 Years of Investigation. Clinical Microbiology Reviews, 15(3), pp.506-526.
4. British Standards Institution (2022) BS7592:2022 Sampling for *Legionella* bacteria in water systems Code of practice. BSI.

Version History*

001 08/03/2023 - New document created
002 24/03/2026 - Removed IVDR status, updated sections 10 and 11 to reflect current QCR

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

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place