Instructions for Use

REF - LS0156 **Legionella BMPA Supplement**

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0156 Legionella BMPA Supplement is an antibiotic supplement used to enhance the selective isolation of *Legionella* species primarily in clinical laboratories.

2. Composition*

For one litre of prepared media

Ingredient	g/L
Cefamandole	0.004
Polymyxin B	80,000IU

*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 [1 2]. Transmission occurs through aerosolization or aspiration of contaminated water, not person to person. Legionellosis refers to two distinct clinical syndromes, Legionnaires' disease, which most often presents as severe pneumonia, and Pontiac fever, which is an acute, febrile, self-limiting, viral-like illness. Over fifty species of *Legionella* have been identified, however *Legionella pneumophila* remains the most prevalent etiological agent of Legionnaires' disease. *Legionella* can be isolated from respiratory tract secretions of all types, sterile body fluids such as blood, and biopsy material.

LS0156 Legionella BMPA Supplement is designed to be used with E&O KM0015 EXP Legionella Agar Export in combination with potassium hydroxide, E&O FX0001 Legionella Supplement Pouch 1 (l-cysteine HCl) and E&O FX0002 Legionella Supplement Pouch 2 (iron (III) pyrophosphate and cycloheximide) to form Legionella BMPA Agar. When made as this final medium, LS0156 is recommended by the UK Standards for Microbiology Investigations [3] and tested in accordance with the principals of ISO 11133:2014 [4].

4. Principle

Legionella BMPA Supplement contains cefamandole and polymyxin B. Cefamandole is a broad-spectrum second generation cephalosporin and is included to inhibit various Gram-positive and Gram-negative bacteria. Polymyxin B is another antibiotic which is included to suppress the growth of Gram-negative bacteria that may interfere with the isolation or identification of *Legionella* species.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water:

Format	Volume of sterile deionised water to add
V002	20ml

Close the vial and gently agitate to assist reconstitution – avoid frothing. The resulting solution will be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium (such as E&O KM0015 EXP Legionella Agar Export with added E&O FX0001 Legionella Supplement Pouch 1 and E&O FX0002 Legionella Supplement 2) prepared according to the instructions provided and allowed to cool to 45°C- 50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	Solid white pellet	Colourless liquid

7. Materials Provided

LS0156 Legionella BMPA Supplement is supplied 10 vials per box (lyophilised). Each bottle is labelled with (abbreviated) product name, product code, lot number, expiry date and as being suitable for addition to the appropriate volume of prepared media. The vials will have freeze dried neutral rubber stoppers and flip off seal caps.

Product code	Product format
LS0156-V002-10L	20ml neutral clear crimped vial suitable for 10L of media

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile deionised water for reconstituting, autoclave, sterile loops or swabs, incubators and quality control organisms.

Ingredients for preparation of media supplied by E&O Laboratories *e.g.*, dehydrated culture medium E&O KM0015 EXP Legionella Agar Export, supplements E&O FX0001 Legionella Supplement Pouch 1 and E&O FX0002 Legionella Supplement Pouch 2.

Ingredients for preparation of media not supplied by E&O Laboratories *e.g.*, potassium hydroxide.

9. Specimens

LS0156 Legionella BMPA Supplement is designed to be used with E&O KM0015 EXP Legionella Agar Export with added potassium hydroxide, FX0001 Legionella Supplement Pouch 1 and E&O FX0002 Legionella Supplement Pouch 2 to form Legionella BMPA Agar, which is suitable for the testing of the following specimens:

- Clinical samples: *Legionella* spp. can be isolated from respiratory tract secretions of all types, body fluids such as blood, and biopsy material.

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.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Based on sample type and information provided, inoculate plated media directly with the sample, or subculture onto plated media after treatment as described in national guidelines.

Clinical Samples [3]

For the isolation of *Legionella* spp. from patients with clinical evidence of Legionnaires' disease, greatest success has been achieved by the examination of lung biopsy tissue and bronchial aspirate.

- Process the clinical specimen as required by local guidelines.
- Inoculate specimens onto Legionella BMPA Agar, and onto non-selective media Legionella BCYE Agar to determine contaminants.
- Incubate the plates at 36 ± 1°C in a 90% relative humidity atmosphere.
    - Incubation in 2-5% CO_2 can enhance growth of some *Legionella* species [3].
- Growth usually appears in 2-3 days but continue to examine daily for 10 days before discarding the plates.

Colonies suspected of being *Legionella* spp. are subcultured to Legionella BCYE Agar and either Legionella BCYE Cysteine Free Agar (E&O PP0201) or a non-selective blood medium.

As the media described are not completely selective for *Legionella* species, it is recommended that the following criteria are used for the examination of plates:

- The colonies isolated must have characteristic colour, size and appearance when examined under a microscope (typical colony appearance outlined in Quality Control table below).
- The isolates should not grow on blood agar or Legionella BCYE Cysteine Free Agar.
- The organisms should show characteristic Gram morphology.

The above points allow for a presumptive identification of *Legionella* species; however, confirmation must be made by further biochemical and serological tests [5]. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
L. pneumophila (NCTC 11192)	37 ± 1°C in 5% CO_2 for 3-5 days	Growth: White colonies
L. anisa (NCTC 11974)	37 ± 1°C in 5% CO_2 for 3-5 days	Growth: White colonies
F. bozemanae (NCTC 11368)	37 ± 1°C in 5% CO_2 for 3-5 days	Growth: White colonies
E. coli (NCTC 12241)	37 ± 1°C in 5% CO_2 for 3-5 days	Inhibited
S. aureus (NCTC 12981)	37 ± 1°C in 5% CO_2 for 3-5 days	Inhibited
S. cerevisiae (NCPF 3178)	37 ± 1°C in 5% CO_2 for 3-5 days	Inhibited
P. aeruginosa (NCTC 12903)	37 ± 1°C in 5% CO_2 for 3-5 days	Inhibited

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

12. Performance

To fully verify LS0156 Legionella BMPA Selective Supplement performance, samples were used to prepare Legionella BMPA Agar and resulting plates 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 -10^5 cfu for the non-target organisms and incubated at 37 ± 1°C in 5% CO_2 for 3-5 days. All samples showed expected recovery and morphology of the required test organisms: *Legionella pneumophila* (NCTC 11192), *Legionella anisa* (NCTC 11974) and *Fluoribacter bozemanae* (NCTC 11368), 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 LS0156 Legionella BMPA Selective Supplement meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- 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.
- Some strains of *L. oakridgensis* may lose the requirement for L-cysteine.
- Incubation in >5% CO_2 can inhibit growth of some *Legionella* species
- Some *Legionella spp.* may be inhibited by cefamandole.

14. 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 LS0156 Safety Data Sheet.

Do not use if supplement is not specified colour, has been stored inappropriately or the packaging has been damaged. Any unused supplement should be discarded.

15. Storage conditions and Shelf life

Legionella BMPA Selective Supplement (LS0156) must be stored at 2-30°C. 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.

16. References

1. 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.
2. Fields, B., Benson, R., and Besser, R. (2002) *Legionella* and Legionnaires' Disease: 25 Years of Investigation. Clinical Microbiology Reviews, 15(3): 506-526.
3. Public Health England (2015) Identification of *Legionella* species. UK Standards for Microbiology Investigations. Bacteriology, 18(3). London: Standards Unit, Microbiology Services.
4. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. Geneva, ISO.
5. Vesey G., Dennis P., Lee J., and West A. (1988) Further development of simple tests to differentiate the *legionellas. J. Appl. Bact.* 65(4), pp.339-345.

Version History*

001 04/04/2019 - Document created.
002 05/04/2019 - Storage temperature changed from 2-8 to 2-30°C
003 22/04/2019 - *Legionella bozemanii* changed to *Fluoribacter bozemanae*. Removed temperature of deionised to be used for dissolving supplement (not required)
004 06/07/2023 - Fully updated IFU to new standard.
005 13/10/2025 - Update to Section 4 to include reconstitution volumes. Clinical sample type methodology added.
006 13/11/2025 - Incubation in 2-5% CO_2 parameters added to test procedure and limitations. can enhance growth of some *Legionella* species. *E. faecalis* removed from Quality Control table and Section 12.

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

EC REP

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place