Instructions for Use

REF - LS0125 **_B. cepacia_ Selective Supplement**

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0125 *B. cepacia* Selective Supplement is a sterile antibiotic supplement used to enhance the isolation of *Burkholderia cepacia* (formerly known as *Pseudomonas cepacia*) on *in vitro* microbial media.

2. Composition*

Ingredient	g/L
Ticarcillin	0.1g
Polymyxin B	300000 IUs

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Accurate quantities of Ticarcillin and Polymyxin B are freeze-dried and provided in individually labelled vials.

Burkholderia cepacia is a gram-negative bacterium found in soil and moist environments that is known to cause various types of infections, including catheter-associated infections and respiratory tract infections. In hospitalized patients. *B. cepacia* can cause pneumonia in immune-compromised patients, especially those with cystic fibrosis. This infection can lead to a rapid decline in those with lung problems and strict isolation is necessary. This is an important opportunistic pathogen in cystic fibrosis patients and can lead to fatal infection in approximately 20% of individuals that have been colonised with *B. cepacia* complex organisms.

When made up as *Burkholderia cepacia* Agar using E&O KM0076: This medium is based on the *Pseudomonas cepacia* medium described by Gilligan *et al*. [(1)] and is recommended by the UK Standards for Microbiology Investigations [(2)] and tested in accordance with ISO 11133:2014 [(3)].

4. Principle

Ticarcillin and polymyxin B are added as supplements to further improve a media's selectivity particularly against *Pseudomonas* spp.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add 2ml of sterile distilled water. Close the vial and gently agitate to assist reconstitution. Avoid frothing and the resulting solution will be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium of choice (e.g., *Burkholderia Cepacia* Agar made up from E&O KM0076) prepared according to the manufacturer's instructions 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	White Solid Pellet	Colourless liquid

7. Materials Provided

E&O Laboratories Ltd *Burkholderia cepacia* Selective Supplement (LS0125) is supplied in 10 x 5ml neutral clear crimped vial suitable for 500ml of media vials per box (lyophilised) (product code: LS0125-V001-500). 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.

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, quality control organisms, and sterile media (e.g., *Burkholderia cepacia* Agar made up from E&O KM0076).

9. Specimens

As LS0125 made up as *Burkholderia cepacia* Agar is suitable for the testing of the following specimens:

- Clinical Specimens: Samples for the testing and isolation of *B. cepacia* may include sputum, specialised swabs from the infected areas, biopsy material, pus, and blood.

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

Clinical Specimens:

- Inoculate specimen directly onto *Burkholderia cepacia* Agar and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate at 35-37°C aerobically for 48-72 hours (additional incubation at 30°C aerobically for a further 5 days may be required).

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

11. Quality Control

Organism	Incubation	Result (Specificity)
B. cepacia (NCTC 10661)	37 ± 1°C aerobically for 24-48 hours	Growth: Green colonies with pink halo
E. coli (NCTC 12241)	37 ± 1°C aerobically for 24-48 hours	Inhibited
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 24-48 hours	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

LS0125 *B. cepacia* Selective Supplement was added to *Burkholderia cepacia* Agar made up from E&O KM0076 following the methods described and 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 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C aerobically for 24-48 hours. All samples showed expected recovery and morphology of the required test organism: *Burkholderia cepacia* (NCTC 10661), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that LS0125 *B. cepacia* Selective Supplement, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

As LS0125 is not to be used independently and is instead designed to be added to an existing medium, limitations will vary depending on the procedure being followed with regards to the medium being used.

14. Precautions and Warnings

This product is considered 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 LS0125 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

E&O Laboratories Ltd *Burkholderia cepacia* Selective Supplement (LS0125) must be stored at 2°-8°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. Gilligan, P., Gage, P., Bradshaw, L., Schidlow, D., and DeCicco, B. (1985) Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol., 22(1), pp.5-8.
2. Public Health England (2015) Identification of *Pseudomonas* species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 17(3). London: Standards Unit, Microbiology Services.
3. 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.

Version History*

001 15/04/15 - New document
002 18/01/17 - Update to new flow diagram format.
003 04/09/18 - Update supplement IFU to addition of 5ml of deionised water from 2ml of water.
004 10/11/23 - Fully updated IFU to new standard

*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	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place