

Instructions for Use

REF - LS0022

Clostridium difficile Selective Supplement Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0022 *Clostridium difficile* Selective Supplement is an antibiotic supplement used to enhance the isolation of *Clostridium difficile* from faecal specimens.

2. Composition* For one litre of prepared media

<u>Ingredient</u>	<u>mg/L</u>
Cefoxitin	8.0
D-Cycloserine	250.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Clostridium difficile can cause severe gastrointestinal infections. It is primarily seen in diarrhoeal disease caused by treatment with antibiotics and is a leading cause of healthcare-associated infections.

Clostridium difficile Selective Supplement is formulated to be used with E&O KM0006 Fastidious Anaerobe Agar with Defibrinated Horse Blood to form *Clostridium difficile* Agar ⁽¹⁾. *Clostridium difficile* Agar is a selective medium for the isolation of *C. difficile* from clinical specimens, and can be used as a selective agar according to the UK Standards for Microbiology Investigations ^(2 3).

4. Principle

Cefoxitin and D-Cycloserine are usually inhibitory to most other clostridial, and related, species and other gut microbiota bacteria. The resulting growth from an active case of infection is often a pure *C. difficile* ⁽²⁾ culture.

5. Preparation Instructions

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

<u>Format</u>	<u>Volume of sterile deionised water to add</u>
V001	5ml

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

Add the contents of the vial to the appropriate volume of sterile base medium, KM0006 Fastidious Anaerobe Agar with Defibrinated Horse Blood, 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

	<u>Pellet Appearance</u>	<u>Reconstituted</u>
<u>Appearance and Colour</u>	White Solid Pellet	Colourless liquid

7. Materials Provided

LS0022 *Clostridium difficile* Selective Supplement is supplied in boxes of 10 vials (lyophilised). Each neutral glass crimped vial is suitable for 500ml of media (product code: LS0022-V001-500). Vials are labelled with product name, product code, lot number and expiry date.

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, quality control organisms, and sterile medium (e.g., *Clostridium difficile* Agar prepared from KM0006 Fastidious Anaerobe Agar with Defibrinated Horse Blood).

9. Specimens

LS0022 *Clostridium difficile* Selective Supplement may be used with the following specimens when added to KM0006 Fastidious Anaerobe Agar with Defibrinated Horse Blood to form *Clostridium difficile* Agar:

- Clinical Specimens: rectal swabs and stool specimens

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:

Follow local, national, or international guidelines.

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 35-37°C anaerobically for 40-48 hours ⁽³⁾. Cultures may be examined after overnight incubation but should not be removed from the anaerobic atmosphere ⁽²⁾.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Typical Gram stain morphology of *C. difficile* may not be evident in colonies picked from this medium because of antibiotics present. Subculture suspected colonies to blood agar to obtain characteristic morphology. Refer to relevant guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>C. difficile</i> (NCTC 11204)	37 ± 1°C anaerobically for 24-48 hours	Growth: Grey colonies
<i>E. coli</i> (NCTC 12241)	37 ± 1°C anaerobically 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

To fully verify LS0022 *Clostridium difficile* Selective Supplement performance, supplement samples were added to sterilised KM0006 Fastidious Anaerobe Agar with Defibrinated Horse Blood 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. Sample plates were inoculated with 30-150cfu and incubated at 37 ± 1°C anaerobically for 24-48 hours. All samples showed good recovery and the correct morphology of the required test organism: *Clostridium difficile* (NCTC 11204). Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10⁴ - ≤1.5x10⁵ cfu of the required non-target test organism: *Escherichia coli* (NCTC 12241), which was inhibited with a selectivity factor of ≥ 3. Therefore, it can be concluded that LS0022 *Clostridium difficile* Selective Supplement meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Medium

- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- Plates of the final selective medium should not be overdried before inoculation.
- Typical Gram stain reaction of *C. difficile* may not be evident in colonies picked from this medium.
- Perform further biochemical, serological, molecular, or mass spectroscopy (MALDI-TOF) testing to confirm identity of presumptive positive isolates. Refer to standard method or relevant local guidelines.

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 may be a potential biohazard if mishandled.

Refer to LS0022 *Clostridium difficile* Selective Supplement Safety Data Sheet prior to use.

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

15. Storage conditions and Shelf life

LS0022 *Clostridium difficile* Selective Supplement should 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. George, W., Sutter, V., Citron, D., and Finegold, S. (1979) Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. 9(2), 214-219.
2. Public Health England (2018) Processing of Faeces for *Clostridium difficile*. UK Standards for Microbiology Investigations. Bacteriology, B 10(1.7). London: Standards Unit, Microbiology Services.
3. Public Health England (2024) Identification of Clostridium species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 8(4.2). London: Standards Unit, Microbiology Services.

Revision

- 001 09/07/2014 - New document
- 002 02/08/2016 - Update with flow diagram
- 003 10/11/2023 - Updated to new format and standard
- 004 28/10/2025 - Update to Section 3 to include reference to SMI, updated Section 5 to include amount of water to reconstitute vials and incubation instructions to reflect SMI

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













E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com



Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

IFU/LS0022 REV. 004

 REF Catalogue number	 Batch code	 <i>In vitro</i> 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