

Instructions for Use

REF - LS1053

Yersinia CIN Selective Supplement **Freeze-Dried Supplement**

1. Intended Use

For *in vitro* diagnostic use. LS1053 Yersinia CIN Selective Supplement is an antibiotic supplement used to enhance the selective isolation and enumeration of *Yersinia* species from clinical and food samples.

2. Composition* For one litre of prepared media

<u>Ingredient</u>	<u>mg/L</u>
Cefsulodin	15.0
Irgasan	4.0
Novobiocin	2.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Yersinia spp. is the causative organism of the infection, yersiniosis. In 1979, Schiemann described a selective and differential medium for isolation of *Y. enterocolitica* which contained cefsulodin, Irgasan, novobiocin, bile salts, and crystal violet as selective agents ⁽¹⁾. He later modified the formula by substituting sodium deoxycholate for bile salts and reduced novobiocin to 2.5 mg/litre, further improving growth and recovery of *Y. enterocolitica* ⁽²⁾.

LS1053 when made up with E&O KM0179 *Yersinia* Selective Agar Base is recommended as a primary isolation media by the UK Standards for Microbiology Investigations ^(3,4) and tested in accordance with ISO 11133:2014 ⁽⁵⁾.

4. Principle

LS1053 Yersinia CIN Selective Supplement contains cefsulodin, Irgasan and novobiocin to enhance the selective isolation of *Yersinia* species by inhibiting Gram-positive and many Gram-negative organisms

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
V001	5ml
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, KM0179 Yersinia Selective Agar, prepared according to the instructions provided and allowed to cool to 45-50°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 LS1053 Yersinia CIN Selective 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
LS1053-V001-500	10 x Vials will have freeze dried neutral rubber stoppers and flip off seal caps, suitable for addition to 500ml of prepared media
LS1053-V002-5000	10 x Vials will have freeze dried neutral rubber stoppers and flip off seal caps, suitable for addition to 5L 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 dehydrated culture medium KM0179 Yersinia Selective Agar.

9. Specimens

LS1053 Yersinia CIN Selective Supplement when made up with E&O KM0179 *Yersinia* Selective Agar Base is suitable for the testing of the following specimens:

- Clinical Specimens: Samples can include faeces and rectal swab ^(2 3 4), as well as samples from the throat, lymph nodes, joint fluid, urine, bile, and blood.
- Food, Water and Environmental: products for human consumption, animal feed ^(6 7) and environmental samples ⁽⁸⁾.

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

If the Yersinia Selective Agar plates produced, using the LS1053 supplement, have been refrigerated, allow to warm to room temperature before inoculation.

Clinical Specimens ^(3 4)

- Process the clinical specimen as required by local guidelines.
- Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates at 28-30°C aerobically for 24-48 hours.

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.

Food, Water and Environmental Specimens ^(6 7 8)

According to ISO 10273:2014, the method required for detecting *Yersinia* spp. is determined by the assumed sample content and may require pre-enrichment in non-selective or selective broths ⁽⁶⁾. Processes requiring membrane filtration techniques may also be used to detect *Yersinia* species ⁽⁸⁾.

- Process the water/environmental sample as required by the local guidelines.
- Inoculate samples onto medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates 30 ± 1°C aerobically for 24 hours.

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.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>Y. enterocolitica</i> (NCTC 12982)	Incubate aerobically at 30 ± 1°C for 24 ± 2 hours	Growth: Clear colonies with red centre
<i>Y. enterocolitica</i> (NCTC 13769)	Incubate aerobically at 30 ± 1°C for 24 ± 2 hours	Growth: Clear colonies with red centre
<i>E. coli</i> (NCTC 12241)	Incubate aerobically at 30 ± 1°C for 24 ± 2 hours	Inhibited
<i>S. aureus</i> (NCTC 12981)	Incubate aerobically at 30 ± 1°C for 24 ± 2 hours	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 relevant local guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify LS1053 Yersinia CIN Selective Supplement performance, supplement samples were added to sterilised KM0179 Yersinia Selective 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 and incubated at 30 ± 1°C for 21 ± 3 hours. All samples showed expected recovery and morphology of the required test organism: *Yersinia enterocolitica* (NCTC 10938). Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10⁴ - ≤1.5x10⁵ cfu of the required non-target test organisms: *Escherichia coli* NCTC 12241 and *Staphylococcus aureus* NCTC 12981, which were inhibited and have a selectivity factor of ≥ 3. Therefore, it can be concluded that LS1053 Yersinia CIN Selective Supplement meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- In case of dense growth of background flora on the Yersinia Selective Agar with CIN plates, the colony size of pathogenic *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent ⁽⁶⁾.
- Other strains of *Yersinia* species, such as *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii* grow equally as well as *Y. enterocolitica* on Yersinia Selective Agar and exhibit the same colony morphology.
- *Serratia* spp., *Enterobacter* spp. and *Citrobacter* spp. are poorly inhibited.

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 LS1053 Safety Data Sheet prior to use.

Do not use if supplement is not 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

E&O Laboratories Ltd LS1053 Yersinia CIN Selective Supplement can be stored at 2 - 8°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Do not use if supplement is not the specified colour. Additionally, do not use if it has been stored inappropriately or the packaging has been damaged.

Dispose of in accordance with local and national authority requirements.

16. References

1. Schiemann, D. (1979) Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. 25, pp.1298-1304.
2. Petersen MJ, Gladney LM, Schrieffer ME. Yersinia. In Jorgensen JH, Carroll KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington DC: American Society for Microbiology; 2015
3. Public Health England (2015) Identification of *Yersinia* species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 21(3). London: Standards Unit, Microbiology Services.
4. Public Health England (2020) Gastroenteritis. UK Standards for Microbiology Investigations. Syndromic, S 7(2). London: Standards Unit, Microbiology Services.
5. 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.
6. International Organization for Standardization (2017) 10273:2017 Microbiology of the food chain - Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Geneva, ISO.
7. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: *Yersinia enterocolitica*. Rev 10/2017
8. The Microbiology of Drinking Water (2002) Part 10 – Methods for the isolation of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment. Standing Committee of Analysts, Environment Agency, Nottingham, UK.

Version History*

001	10/06/2015 - New document created
002	02/03/2016 - Update to new marketing format PDF
003	27/07/2016 - Update to new flow-based diagram
004	28/09/2017 - Adjust instruction to rehydrate in 5mls water
005	05/06/2019 - Added a TDS option for 5 litre vials
006	06/07/2023 - Fully updated IFU to new standard
007	13/10/2025 - Update to Section 4 to include reconstitution volumes
008	09/01/2026 - Fully revised to reflect Product Device File and latest revision of the Quality Control Record

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



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IFU/LS1053 REV. 008

TABLE OF APPLICABLE SYMBOLS

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