

Instructions for Use

Campylobacter Growth Supplement

Freeze-Dried Supplement

REF - LS0011

1. Intended Use

For *in vitro* diagnostic use only. LS0011 Campylobacter Growth Supplement is used to enhance the aerotolerance and isolation of *Campylobacter* spp.

2. Composition* For one litre of prepared media

<u>Ingredient</u>	<u>mg/L</u>
Sodium metabisulphite	250
Iron sulphate	250
Sodium pyruvate	250

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS0011 Campylobacter Growth Supplement is formulated for use with KM0001 Columbia Agar Base, HBL Horse Blood Lysed, and Skirrow Selective Supplement to form Campylobacter Selective Agar (Skirrow).

Campylobacter Selective Agar (Skirrow) is a selective media available for the isolation of *Campylobacter* spp. from clinical, food and environmental samples. *Campylobacter* spp. are Gram-negative, spiral-shaped microaerophilic organisms found in raw milk, untreated water, improperly handled food, raw meats, poultry, and shellfish. Infection with these organisms can cause a variety of clinical illnesses, ranging from transient asymptomatic colonisation to severe dysentery. *Campylobacter enteritis* symptoms include diarrhoea, stomach pain, nausea, fever, headache, and muscle pain.

Campylobacter Selective Agar (Skirrow) is based on the original Columbia Agar formulation described by Ellner *et al.* from Columbia University ⁽¹⁾. The medium is further enriched with lysed horse blood, sodium metabisulphite, sodium pyruvate and iron (II) sulphate to improve *Campylobacter jejuni* recovery and morphology ⁽²⁾. Additionally, this medium is formulated to allow recovery at both 37°C and 42°C ⁽³⁾ incubation, is recommended by the UK Standard for Microbiology Investigation for the isolation of *Campylobacter* spp. from clinical specimens such as faeces ⁽⁴⁾ and is tested in accordance with the principals of ISO 11133:2014 ⁽⁵⁾.

4. Principle

Accurate quantities of sodium metabisulphite, sodium pyruvate and iron (II) sulphate are used to enhance the isolation and aerotolerance of *Campylobacter* spp.

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

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, KM0001 Columbia Agar Base, Skirrow Selective Supplement and lysed horse blood prepared according to the instructions provided and cooled to 45-50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	Beige/Off-White Solid Pellet	Beige/Yellow liquid

7. Materials Provided

LS0011 Campylobacter Growth Supplement is supplied in boxes of 10 vials (lyophilised). Each vial is labelled with product name, product code, lot number and expiry date.

Product code	Product format
LS0011-V001-500	5ml neutral clear crimped vial suitable for 500ml of media

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms, sterile media (e.g., Campylobacter Selective Agar (Skirrow) made up from KM0001 Columbia Agar Base, HBL Horse Blood Lysed, and Skirrow Selective Supplement)

9. Specimens

LS0011 Campylobacter Growth Supplement can be used with the following specimens when added to KM0001 Columbia Agar Base, HBL Horse Blood Lysed, and Skirrow Selective Supplement to form Campylobacter Selective Agar (Skirrow):

- Food Industry: Products for human consumption, animal feed and environmental samples.
- Clinical: Rectal swabs and stool specimens, body tissue, or fluids.

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

Follow local, national, or international guidelines.

Allow prepared plates to come to room temperature.

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Food and Environmental Specimens:

- Pre-enrich or process food/environmental sample as required by relevant guidelines.
- Prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760).
- Inoculate homogenised sample or pre-enriched sample directly onto agar.
- Incubate at $37 \pm 1^\circ\text{C}$ microaerophilically for 44 ± 4 hours.

Clinical Specimens:

- Process clinical specimen as required by relevant 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 $42 \pm 1^\circ\text{C}$ microaerophilically for 44 ± 4 hours.

After incubation, examine plates for typical colonies. 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>C. jejuni</i> (NCTC 11322)	$37 \pm 1^\circ\text{C}$ microaerophilically for 48 hours	Growth: Grey spreading colonies

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 the performance of LS0011 Campylobacter Growth Supplement, supplement samples were added to sterilised KM0001 Columbia Agar Base and lysed horse blood and resulting plates tested to assess colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) compared to a Columbia agar with lysed horse blood plate with no growth supplement. Samples were inoculated with 30-150cfu and incubated $37 \pm 1^\circ\text{C}$ microaerophilically for 48 hours. All samples showed good recovery and the correct morphology of the required test organism: *Campylobacter jejuni* (NCTC 11322). Therefore, it can be concluded that LS0011 Campylobacter Growth Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to nutritional variation, some strains may grow poorly or fail to grow on the final medium.
- Plates of the final selective medium should not be overdried before inoculation.
- Perform further biochemical, serological 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 should be regarded as a potential biohazard if mishandled.

Refer to LS0011 Campylobacter Growth 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

LS0011 Campylobacter Growth Supplement must be stored at $2^\circ\text{--}8^\circ\text{C}$. 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. Ellner, P., Stoessel, C., Drakeford, E. and Vasi, F. (1966) A New Culture Medium for Medical Bacteriology. American Journal of Clinical Pathology, 45: 502-504.
2. Chou, S.P., Dular, R. and Kasatiya, S. (1983) Effect of ferrous sulfate, sodium metabisulfite, and sodium pyruvate on survival of *Campylobacter jejuni*. J. Clin. Microbiol., 18(4): 986-987.
3. Skirrow, M.B. (1977) British Medical Journal 2: 9-11.
4. Public Health England. (2018). Identification of *Campylobacter* species. UK Standards for Microbiology Investigations. ID 23 Issue 3.1.
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.

Version History*

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|-----|---|
| 001 | 08/07/2014 - New Document |
| 002 | 28/01/2015 - Addition of new logo and add information about 1Lt vials. |
| 003 | 23/06/2016 - Update to new format and also add word document for 500ml. |
| 004 | 01/08/2016 - Update to new format with diagram. |
| 005 | 04/12/2023 - Updated to new format and standard |
| 006 | 01/02/2024 - IVD symbol and statement added. Relevant clinical processes referenced |
| 007 | 13/10/2025 - Update to Section 4 to include reconstitution volumes. |

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



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TABLE OF APPLICABLE SYMBOLS

IFU/LS0011 REV. 007

 REF Catalogue number	 LOT Batch code	 IVD <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