INSTRUCTIONS FOR USE

CAMPYLOBACTER GROWTH SUPPLEMENT

Freeze-dried supplement

1 - INTENDED USE

In vitro diagnostic. Supplement for improving the growth and aerotolerance of *Campylobacter* spp.

2 - COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Sodium pyruvate	125 mg
Sodium metabisulphite	125 mg
Ferrous sulphate	125 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter Growth Supplement is a freeze-dried mixture of compounds prepared according to the formulation devised by George at al.[1] Sodium pyruvate, sodium metabisulphite and ferrous sulphate, added to plating media or liquid enrichment media, enhance the isolation and the oxygen tolerance of *Campylobacter* spp. by quenching superoxide anions and hydrogen peroxide which occur spontaneously in the culture medium.[2,3]

The complete medium may be prepared by adding Campylobacter Growth Supplement to a medium base such as Columbia Agar Base, Nutrient Broth n° 2, with 5-7% lysed defibrinated horse or sheep blood, and the rehydrated contents of one vial of a selective supplement such as Skirrow, Butzler, Preston or Blaser Wang.

4- DIRECTIONS

Aseptically reconstitute the content of one vial of Campylobacter Growth Supplement with 5 mL of sterile purified water and mix gently to dissolve.

Prepare 500 mL of the suitable medium base, autoclaved and cooled to 47-50°C. Add the supplement together with 25-35 mL of lysed defibrinated horse or defibrinated sheep blood and, if required, with the rehydrated contents of one vial of a selective supplement. Mix well and pour into sterile Petri dishes or sterile tubes.

Suggested basal media and selective supplements include: Columbia Agar Base (401136), Nutrient Broth n° 2 (401812), Skirrow Antimicrobic Supplement (4240016), Preston Antimicrobic Supplement (4240017, 4240022).

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	short, friable, grey-green pastille
Reconstituted supplement appearance	grey-green, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Growth Supplement	Freeze-dried supplement	4240021	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Suitable medium base and selective supplement, lysed horse or sheep blood, autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes and tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies. Materials for incubation in a microaerophilic atmosphere.

8 - SPECIMENS

Complete media prepared with Campylobacter Growth Supplement are intended for the bacteriological processing of clinical specimens such as faeces and rectal swab[4,5] and non-clinical specimens such as food and animal feeding stuffs. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

- Solid faeces: faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[3] Inoculate 3-5 drops on the medium surface.
- Liquid stool: inoculate 3 drops on the medium surface.
- Rectal swabs: roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

For all type of specimens, streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Incubate in a microaerobic atmosphere consisting approximately of 5% O_2, 10% CO_2, and 85% N_2, at 39-42°C for 40-48 hours.[2]

For the detection of *Campylobacter* in food samples, refer to the applicable Standards.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Colonies of *C. jejuni* usually are grey/white or creamy grey in colour, swarming and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.

Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacter* species is oxidase negative, subculture to blood agar and retest after 24hr incubation.[6]

The presumptive identification of thermophilic and enteropathogenic *Campylobacter* can be done on the basis of oxidase test (+) and the characteristic motility. For a complete explanation of the identification criteria and methods, refer to the quoted reference.[6]

Plates examined after 24 h of incubation should be rapidly examined and re-incubated under microaerophilic conditions to maintain

viability of the strains most sensitive to oxygen.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, , the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.jejuni	ATCC 33291	39-42°C / 40-48h / M	good growth
C.coli	ATCC 43478	39-42°C / 40-48h / M	good growth
E.coli	ATCC 25922	39-42°C / 40-48h / M	partially or totally inhibited
S.aureus	ATCC 25923	39-42°C / 40-48h / M	inhibited

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Campylobacter Growth Supplement added to dehydrated Columbia Agar Base n° 2 Base together with 5% of lysed horse blood and Skirrow Antimicrobic Supplement, is tested for productivity and selectivity by comparing the results with previously approved Reference Batch.
Productivity is tested by a semi-quantitative test with the target strains *C.jejuni* ATCC 33291 and *C.coli* ATCC 43478 ; plates are inoculated with decimal dilutions in saline of the colonies suspensions and incubated in a microaerophilic atmosphere at 39-42°C for 24 hours. Both strains show a good growth.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *E.coli* ATCC 25922 and *S.aureus* ATCC 25923 The growth of *S.aureus* and is totally inhibited while the growth of E.coli is partially inhibited after incubation at 39-42°C for 48 hours in a microaerophilic atmosphere.

13 - LIMITATIONS OF THE METHOD

- To achieve the highest yield of *Campylobacter* from stool samples, a combination of media that includes Skirrow medium and a second selective medium, based on a different selective system, appears to be the optimal method (e.g., Karmali medium).[7]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[4] Enrichment seems not to be necessary for samples collected in the acute campylobacteriosis phase, while *Campylobacter* recovery increases in asymptomatic patients, in studies involving low numbers of the target organism, in samples not readily sent to the laboratory and in samples taken in the convalescence phase after an episode of diarrhea.[7,8]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The culture medium and the supplement are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The supplement id classified as dangerous according to European rules; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- The supplement is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics). The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the types (plates/tubes) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. George HA, Hoffman PS, Smibert RM, Krieg NR. Improved media for growth and aerotolerance of Campylobacter fetus. J.Clin Microbiolv.8(1); 1978.
2. Hoffman PS, George HA, Krieg NR, Smibert RM. Studies of the microaerophilic nature of Campylobacter fetus subsp. jejuni. II. Role of exogenous superoxide anions and hydrogen peroxide. Can J Microbiol 1979 Jan;25(1):8-16.

3. Chou SP, Dular R, Kasatiya S. Effect of ferrous sulfate, sodium metabisulfite, and sodium pyruvate on survival of Campylobacter jejuni. J Clin Microbiol. 1983 Oct;18(4):986-7
4. Fitzgerard C, Nachamamkin I. Campylobacter and Arcobacter. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.998
5. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
6. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018
7. Endtz HP, Ruijs GJ, et al. Comparison of six media including a semisolid agar for the isolation of various Campylobacter species from stool specimens. J Clin Microbiol 1991; 29:1007
8. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462

4240021 CAMPYLOBACTER GROWTH SUPPLEMENT
SDS
Regulation (EU) 2020/878

Mixture with hazardous ingredients: SODIUM BISULPHITE, SODIUM PIRUVATE

Classification

Serious eye damage, category 1	H318	Causes serious eye damage.
Skin irritation, category 2	H315	Causes skin irritation.
Skin sensitization, category 1B	H317	May cause an allergic skin reaction.

Labelling

Pictogram

Signal word Warning

Hazard statement(s)

H318	Causes serious eye damage.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
EUH031	Contact with acids liberates toxic gas.

Precautionary statement(s)

P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P280	Wear protective gloves / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor / . . .
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P264	Wash . . . thoroughly after handling.
P362+P364	Take off contaminated clothing and wash it before reuse.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/02
Revision 2	Removal of obsolete classification	2023/04

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