INSTRUCTIONS FOR USE

KARMALI ANTIMICROBIC SUPPLEMENT

Freeze-dried selective supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of antimicrobials to be added to Campylobacter Blood Free Medium Base (Karmali) for the isolation of thermotolerant *Campylobacter* spp. from clinical and other specimens.

2 - COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefoperazone	16 mg
Vancomycin	10 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Karmali Antimicrobic Supplement is a freeze-dried mixture of antimicrobials to be used as a supplement of Campylobacter Blood Free Medium Base (Karmali). The complete medium Karmali Medium, originally devised by Karmali in 1986,[1] is a selective medium for the isolation of thermotolerant *Campylobacter* spp. from faeces and other non-clinical samples.
The selective agents of the supplement are vancomycin, with a strong inhibitory activity against Gram positive bacteria and cefoperazone, which mainly suppresses the growth of Gram-negative bacteria. Cycloheximide is included as an antifungal compound in the basal medium. Charcoal (in substitution of animal blood), haematin and sodium pyruvate of the basal medium stimulate the growth of *Campylobacter*, increase its aero tolerance and inhibit the toxic compounds that are formed during the growth.

4- DIRECTIONS

Aseptically reconstitute the content of one vial with 5 mL of sterile purified water and mix gently to dissolve.
Prepare 500 mL of Campylobacter Blood Free Medium Base (Karmali) (401283) autoclaved at 115°C for 15 minutes and cooled to 47-50°C. Add the contents of one vial of Karmali Antimicrobic Supplement under aseptic conditions, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	short, dense, white pastille
Reconstituted supplement appearance	limpid, colourless

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Karmali Antimicrobic Supplement	Freeze-dried supplement	4240035	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Campylobacter Blood Free Medium Base (Karmali) (401283), autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies. Materials for incubation in a microaerophilic atmosphere.

8 - SPECIMENS

Faecal specimens are preferred for isolating *Campylobacter* spp. from patients with gastrointestinal infections; however, rectal swabs are acceptable for culture.[2] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For non-clinical samples, refer to the applicable international standards.

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]

10 - READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies 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.[4]
The presumptive identification of thermophilic and enteropathogenic Campylobacter can be done on the basis oxidase test (+) and the characteristic motility.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[4]

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.

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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

A: aerobic incubation; 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 Karmali Antimicrobic Supplement added to dehydrated Campylobacter Blood Free Medium Base Karmali,, is tested for productivity and selectivity by comparing the results with previously approved Reference Batches.
Productivity is tested by a quantitative test with the target strains *C.coli* ATCC 43478 and *C.jejuni* ATCC 33291; Karmali plates are inoculated with decimal dilutions in saline of the colonies suspensions and incubated at 39-42°C for 40-48 hours in microaerobic atmosphere. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio (*Pr*=CFU_{TB}/CFU_{RB}) is calculated. If *Pr* is ≥ 0.7 the results are considered acceptable and conform to the specifications.
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 *C.albicans* ATCC 18804, *E.coli* ATCC 8739, *S.aureus* ATCC 25923, *E.faecalis* ATCC 19433. *C.albicans* is partially inhibited, the growth of other non-target strains is totally inhibited after incubation of 72 hours at 39-42°C in microaerobic atmosphere.
Campylobacter Blood Free Agar (Karmali) plates, prepared as described above, were compared with CCDA Preston medium by Varoli et al.[5] with 198 stool specimens. *Campylobacter* spp. was recovered in 8 samples on both media but on Karmali medium 5 isolates have been found in pure culture, while on CCDA Preston it was found with only 2 isolates. No significant differences were found between the two media as regards the growth of microbial contaminating flora of yeasts and Gram-negative bacilli; Karmali medium has been evaluated more inhibitory for the Gram-positive bacteria growth.

13 - LIMITATIONS OF THE METHOD

- The most numerous contaminants found in the Karmali medium are *Enterobacteriaceae*, which are resistant to cefoperazone when present in high numbers, especially *Klebsiella oxytoca*.[6]
- To achieve the highest yield of *Campylobacter* from stool samples, a combination of media that includes Karmali medium and a second selective medium, based on a different selective system, appears to be the optimal method (e.g., Skirrow medium).[7]
- Extending the incubation time from 48 to72 h leads to an increase in the isolation rate.[7]
- Blood free formulations (e.g., Karmali, CCDA) appear to have better performances than blood containing media.[2]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[2] 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.[8,9]
- 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.
- Antibiotics containing supplements must be handled with suitable protection; 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 preparation process of plated 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 plated media and the validation of their shelf life, according to the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S., Lane, J. (1986) J. Clin. Microbiol. 21, 456-59
2. 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.
3. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
4. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018
5. Varoli, O., Gatti M. (1989) Personal communication.
6. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *in* Handbook of Culture Media for Food and Water Microbiology, Edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
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
9. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? J Clin Pathol 1983; 36:1350-1352

KARMALI ANTIMICROBIC SUPPLEMENT **4240035**
SDS rev 5
Regulation (EU) 2020/878

Contains: cefoperazone, vancomycin HCl

Classification

Respiratory sensitization, category 1	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

Hazard statement(s)

H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317	May cause an allergic skin reaction.

Precautionary statement(s)

P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
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	2022/03
Revision 2	Removal of obsolete classification	2023/04

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

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