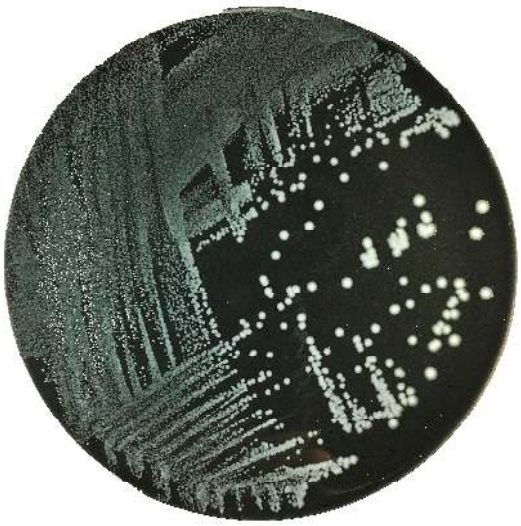
INSTRUCTIONS FOR USE

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (KARMALI)

KARMALI ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement



Campylobacter coli on Campylobacter Blood Free Medium (Karmali),

1 - INTENDED USE

In vitro diagnostic. Basal medium and selective supplement for the isolation of thermotolerant *Campylobacter* spp. from clinical and other specimens.

2 - COMPOSITION

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (KARMALI)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptocomplex	10.000 g
Tryptose	10.000 g
Peptone	3.000 g
Maize starch	1.000 g
Sodium chloride	5.000 g
Charcoal	4.000 g
Haematin	0.032 g
Sodium pyruvate	0.100 g
Cycloheximide	0.100 g
Agar	14.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

KARMALI ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cefoperazone	16 mg
Vancomycin	10 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter spp. are Gram-negative, oxidase-positive, non-sporeforming, S-shaped, or spiral rods, 0.2–0.9 µm wide and 0.5–5 µm long. Organisms are usually motile by means of a single polar unsheathed flagellum at one or both ends, that gives them a very characteristic “corkscrew” motility.[1] An atmosphere containing reduced oxygen (5 to 6%) is required for microaerobic growth. The species most commonly associated with disease in humans are thermotolerant: they will grow at 42°C - 43°C and 37°C, but not at 25°C. *Campylobacter jejuni* subspecies *doylei*, *Campylobacter fetus* and *C. fetus* subspecies *venerealis* do not grow at 42°C.[2]

In *Campylobacter* infection (campylobacteriosis), the symptoms usually range from none to severe, including fever, abdominal cramping, and diarrhoea (with or without blood/faecal white cells); nausea and vomiting may accompany the diarrhoea. Extraintestinal infections have been reported following *Campylobacter* enteritis in less than 0.15% of patients, usually in very old or very young subjects, and include bacteraemia, hepatitis, pancreatitis, meningitis, endocarditis, septic arthritis, abortion, neonatal sepsis; *C.jejuni* is the most often recognized infection preceding the development of Guillain-Barré syndrome.[1]

Campylobacter infections are acquired by ingestion of undercooked poultry, seafood, meat and produce, by the contact with animals and by drinking untreated water or milk. Most infections are caused by *C. jejuni* subsp. *jejuni* and *C. coli*: other species which sometimes cause diarrhoea are *C.lari*, *C.fetus subsp. fetus*, *C.jejuni subsp. doylei* and *C.upsaliensis*.

Since the early 1970s, when *C.jejuni* and *C. coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[3] The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixture of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[3] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.

Campylobacter Blood Free Medium Base Karmali and the selective supplement Karmali Antimicrobic Supplement are prepared according to the formulation devised by Karmali in 1986.[4] Campylobacter Blood Free Medium Karmali and Karmali Supplement are intended for the isolation of thermotolerant *Campylobacter* spp. from faeces and other non-clinical samples.

The medium of Karmali *et al*. is a variation of mCCDA of Bolton, Hutchinson and Coats[5], using haematin rather than ferrous sulphate, vancomycin instead of sodium deoxycholate and cycloheximide instead of amphotericin B.

The selective agents of the medium are vancomycin, with a strong inhibitory activity against Gram positive bacteria, cefoperazone, which mainly suppresses the growth of Gram-negative bacteria and cycloheximide, included as an antifungal compound. Charcoal (in substitution of animal blood), haematin and sodium pyruvate stimulate the growth of *Campylobacter*, increase its aero tolerance and inhibit the toxic compounds that are formed during the growth.

Karmali Medium (KM) was compared to Skirrow medium (SKM) for the recovery of *C.jejuni* and *C.coli* from stool of patients with diarrhea.[3] These campylobacters were isolated from 35 (2.9%) of 1,227 stools tested (29 on both media, 5 on KM alone, and one on SKM alone). Whenever *C.jejuni* and *C.coli* were recovered, growth was pure on 29 KM cultures (85%), but on only 11 SKM cultures (37%). Complete suppression of "contaminating" flora occurred in 704 KM cultures (57%) compared with 426 SKM cultures (35%).

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4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24.1 g in 500 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 118°C for 15 minutes and cool to 47-50°C. Add the contents of one vial of Karmali Antimicrobic Supplement (REF 4240035) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Campylobacter Blood Free Medium Base Karmali

Dehydrated medium appearance	black, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	black opaque
Final pH at 20-25 °C	7.4 ± 0.2

Karmali Antimicrobic Supplement

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Free Medium Base Karmali	Dehydrated medium	4012832	500 g (10.4 L)
Karmali Antimicrobic Supplement	Freeze-dried supplement	4240035	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Faecal specimens are preferred for isolating *Campylobacter* spp. from patients with gastrointestinal infections; however, rectal swabs are acceptable for culture.[3] 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.[2] 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.[6]
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.[6]

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 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

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 dehydrated Campylobacter Blood Free Medium Base Karmali, supplemented with Karmali Antimicrobic Supplement, 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 42°C for 24-72 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.

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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.
Campylobacter Blood Free Agar (Karmali) plates, prepared as described above, were compared with CCDA Preston medium by Varoli et al.[7] 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*.[1]
- 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).[8]
- Extending the incubation time from 48 to72 h leads to an increase in the isolation rate.[8]
- Blood free formulations (e.g., Karmali, CCDA) appear to have better performances than blood containing media.[3]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[3] 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.[9,10]
- 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 medium base and the supplement are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- The supplement is sterilised by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and 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 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 products for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at 2-8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method applied (temperature and packaging).

Selective supplement

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 type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. 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.
2. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
3. 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.
4. Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S., Lane, J. (1986) J. Clin. Microbiol. 21, 456-59
5. Bolton FJ, Hutchinson DN, Coates D. A blood-free selective medium for the isolation of C.jeuni from faeces. J Clin Microbiol 1984; 19:169.
6. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018
7. Varoli, O., Gatti M. (1989) Personal communication.
8. 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
9. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462
10. 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	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Updated layout and content	2020/09
Revision 4	Update of “precautions and warnings”, storage conditions and shelf life” and signals of danger	2022/04
Revision 5	Removal of obsolete classification	2023/04

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