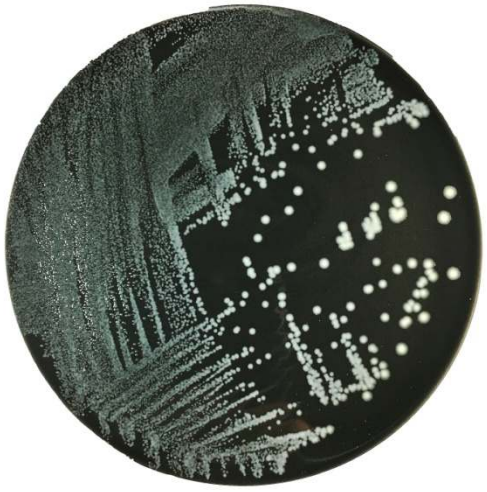
INSTRUCTIONS FOR USE

CAMPYLOBACTER BLOOD FREE AGAR KARMALI

Ready-to-use plates



Campylobacter coli on
Campylobacter Blood Free Agar (Karmali)

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of thermotolerant *Campylobacter* spp. from clinical and other specimens.

2 - COMPOSITION - TYPICAL FORMULA *

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
Cefoperazone	0.032 g
Vancomycin	0.020 g
Purified water	1000 mL

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

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 42-43°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 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 1970’s, 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 Agar Karmali is prepared according to the formulation devised by Karmali in 1986[4] and is 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%).

4 - PHYSICAL CHARACTERISTICS

Medium appearance	black opaque
Final pH at 20-25 °C	7.4 ± 0.2

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5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Free Agar Karmali	Ready-to-use plates	541136	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - 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.

8 - 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]

9 - 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 of oxidase test (+) and the characteristic motility.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[6]

10 - 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

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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all ready-to-use plates of Campylobacter Blood Free Agar Karmali and of the raw materials used for the production of prepared plates (dehydrated Campylobacter Blood Free Medium Base Karmali, REF 401283, supplemented with Karmali Antimicrobic Supplement, REF 4240035), are 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° 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 25823, *E.faecalis* ATCC 19433. After incubation at 39-42° for 72 hours *C.albicans* is partially inhibited, the growth of other non-target strains is totally inhibited.
Campylobacter Blood Free Agar Karmali was 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.

12 - 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.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to-use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - 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.jejuni 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

TABLE OF APPLICABLE SYMBOLS

REF or 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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 2	Updated layout and content in compliance with IVDR 2017/746	2020/08
Revision 3	Removal of obsolete classification	2023/03

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

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