m-CCDA Agar
CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON (m-CCDA)
BOLTON CCDA ANTIMICROBIC SUPPLEMENT
CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON)

Dehydrated and ready-to-use culture medium and selective supplement

1 - INTENDED USE
Selective medium for the isolation of *Campylobacter* spp. in foodstuffs and other samples.

2 – COMPOSITION*
CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON -DEHYDRATED MEDIUM TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	10.00 g
Peptone	10.00 g
Tryptone	3.00 g
Sodium chloride	5.00 g
Charcoal	4.00 g
Sodium deoxycholate	1.00 g
Ferrous sulphate	0.25 g
Sodium pyruvate	0.25 g
Agar	15.50 g

BOLTON CCDA ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefoperazone	16 mg
Amphotericin B	5 mg

CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON) - READY TO USE PLATES

Campylobacter Blood Free Medium Base Bolton	49 g
Cefoperazone	32 mg
Amphotericin B	10 mg
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
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.[1]
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[1] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.
Blood free formulations (e.g., mCCDA, Karmali) appear to have better performances than blood containing media.[2]
Campylobacter Blood Free Medium CCDA Bolton is prepared according the formulation proposed by Bolton, Hutchinson and Coates[3] and later modified by the replacement of cefazolin with cefoperazone to improve the selectivity properties.[4] The medium is recommended by ISO 10272[5,6] for detection and enumeration of *Campylobacter* spp. in samples of the food chain and by ISO 17995[7] for detection and enumeration of thermotolerant *Campylobacter* spp, in water. It is included by APHA[8] and FDA-BAM[9] in the range of selective isolating agars for the detection of *Campylobacter* in food.
The medium is known also as “modified charcoal cefoperazone deoxycholate agar (mCCD agar)”
Beef extract, tryptone and peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. Charcoal, sodium pyruvate and ferrous sulphate, enhance the isolation and the oxygen tolerance of *Campylobacter* spp. by quenching superoxide anions and hydrogen peroxide which occur spontaneously in the culture medium[10]; sodium chloride maintains the osmotic balance. The selective agents of the medium are: sodium desoxycholate active against Gram-positive bacteria, cefoperazone which mainly suppresses the growth of Gram-negative bacteria and amphotericin B, included as an antifungal compound.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION
Suspend 24.5 g of Campylobacter Blood Free Medium Base Bolton in 500 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes and cool to 47-50°C. Add the contents of one vial of Bolton CCDA Antimicrobic Supplement (REF 4240020) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS
Campylobacter Blood Free Medium Base Bolton

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

Bolton CCDA Antimicrobic Supplement

Freeze-dried supplement appearance	short, yellowish pastille
Reconstituted supplement appearance	pale yellow, opalescent

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6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Free Medium Base Bolton (m-CCDA)	Dehydrated medium	4012822	500 g (10.2 L)
Bolton CCDA Antimicrobic Supplement	Freeze-dried supplement	4240020	10 vials, each for 500 mL of medium
Campylobacter Blood Free Agar (CCDA Bolton)	Ready-to-use plates	541113	2 x 10 plates ø 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, sterile membrane filters, Erlenmeyer flasks, controlled atmosphere generators and jars, ancillary culture media and reagents.

8 - SPECIMENS

Water, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards[5-7] for the collection, transport, storage and preparation of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

According to ISO 10272-1, depending on the type of sample and the purpose of the test, three different detection procedures of *Campylobacter* can be used:

A. detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters: enrichment in Bolton broth* with incubation in a microaerobic atmosphere at 37°C for 4 h to 6 h and then at 41.5 °C for 44 h.
B. detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and high level of background microflora: enrichment in Preston broth^ with incubation in a microaerobic atmosphere at 41.5 °C for 24 h.
C. detection of *Campylobacter* by direct plating, in samples with high numbers of campylobacters.

Detection procedure A: from the enrichment culture in Bolton Broth, inoculate two selective solid media:

- mCCD Agar
- any other solid selective Campylobacter medium using a different selective principle.

Detection procedure B: from the enrichment culture in Preston Broth inoculate the plates of mCCDA agar.

Detection procedure C: the test portion is plated directly or after suspending in an appropriate amount of liquid onto the plates of mCCD agar.

The selective isolation agars are incubated at 41.5 °C in a microaerobic atmosphere and examined after 44 h to detect the presence of suspect *Campylobacter* colonies.

In general, the detection of *Campylobacter* in water according to ISO 17995 requires enrichment followed by isolation of colonies and their confirmation.[7] Samples with expected high contamination levels are inoculated directly into Preston broth; where the expected level of background microorganisms is low and samples cannot be processed by membrane filtration, Bolton broth may be used. If no information about the contamination level is available, both broths should be used. The incubated enrichment broths are inoculated onto mCCDA plates and incubated at 41.5 ± 1 °C for 44 ± 4 h.

*Bolton broth: Campylobacter Bolton Broth Base REF 401286B2 + Bolton Broth Selective Supplement REF 4240025.
^Preston broth: Nutrient Broth n° 2 REF 401812S2 + Preston Antimicrobic Supplement REF 4240022 + Lysed Horse Blood REF 90HLX100.

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 greyish on mCCD Agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies tend to spread less on drier agar surfaces. Other forms of colonies may occur.
The suspect *Campylobacter* colonies are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase activity and an aerobic growth test at 25°C. Optionally, the *Campylobacter* species are identified by specific biochemical tests and/or molecular methods.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[5-9]

11 - USER QUALITY CONTROL

All manufactured lots of the products 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	40.5-42.5°C / 40-48h / M	good growth
C. coli	ATCC 43478	40.5-42.5°C / 40-48h / M	good growth
E. coli	ATCC 25922	40.5-42.5°C / 40-48h / M	inhibited
S. aureus	ATCC 25923	40.5-42.5°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 representative samples of all lots of dehydrated Campylobacter Blood Free Medium Base Bolton supplemented with Bolton CCDA Antimicrobic Supplement and of ready-to-use plates of Campylobacter Blood Free Agar CCDA Bolton, are tested for productivity and selectivity by comparing the results with previously approved Reference Batches and Tryptic Soy Agar (TSA).
Productivity is tested by a quantitative test with the target strains *C. coli* ATCC 43478 and *C. jejuni* ATCC 29428; mCCDA agar plates are inoculated with decimal dilutions in saline of the colonies suspensions and incubated at 42°C for 40-48 hours in microaerobic atmosphere. The colonies are enumerated on Test Batch (TB) and TSA and the productivity ratio (Pr=CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 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 while the growth of other non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- The most numerous contaminants found in the mCCD agar medium are *Enterobacteriaceae*, which are resistant to cefoperazone when present in high numbers, especially *Klebsiella oxytoca*.[1]
- 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.

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplement and the ready-to-use plates are for microbiological control and 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 must be handled with suitable protection. Bolton CCDA Antimicrobic Supplement is classified as dangerous. 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.
- Be careful when opening the metal ring of vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- 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.
- 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 Sheets are available on the website www.biolifeitaliana.it.
- 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

Ready to use plates

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).

Dehydrated medium

Upon receipt, store at +10°C /+30°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).

Freeze-dried 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/bottles) and the applied storage conditions (temperature and packaging). According to ISO 17995 the self-prepared mCCDA plates can be stored at 5 ± 3 °C in airtight bags, in the dark for not more than 10 days, while the medium base can be stored in airtight bottles, in the dark, at 5 ± 3 °C for not more than one month.[7]

16 - REFERENCES

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5. ISO 10272-1:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method
6. ISO 10272-2:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique
7. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp
8. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Keep away from direct light	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/06
Revision 2	Update table of symbols	2024/02

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

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