



## PRESTON MEDIUM CAMPYLOBACTER BLOOD AGAR BASE PRESTON ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement



*Campylobacter jejuni*  
on Preston medium

### 1 - INTENDED USE

Basal medium and selective supplement for the isolation of thermotolerant *Campylobacter* spp.

### 2 - COMPOSITION

#### CAMPYLOBACTER BLOOD AGAR BASE

#### TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) \*

Beef Extract	10 g
Peptone	10 g
Sodium Chloride	5 g
Agar	15 g

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

#### PRESTON ANTIMICROBIC SUPPLEMENT

#### (VIAL CONTENT FOR 500 ML OF MEDIUM)

Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim	5 mg
Polymyxin B	2.500 U.I.

### 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.<sup>1</sup> 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.<sup>2</sup>

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.<sup>1</sup>

*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.<sup>3</sup> The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixtures of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay<sup>3</sup> mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.

Preston medium, is prepared according to the formulation described by Bolton and Robertson<sup>2</sup> and consists of *Campylobacter* Blood Agar Base with the addition of Preston Antimicrobial Supplement and lysed horse blood. *Campylobacter* Blood Agar Base is essentially a Nutrient Broth n° 2 with the addition of agar and was chosen by Bolton and Robertson for its low content in trimethoprim inhibitors.<sup>2</sup>

A comparison of Skirrow's, Butzler's, Blaser's, Campy-BAP and Preston media for *Campylobacter* spp. was made by Bolton *et al.*<sup>3</sup> using human, animal and environmental specimens. Butzler's medium gave the lowest isolation rate and Preston medium, which was the most selective, the highest isolation rate. Enrichment culture using Preston enrichment broth gave a higher isolation rate than direct plating onto Preston medium. Therefore Preston medium can be used for the isolation of *C.jejuni* and *C.coli*, from specimens of human origin, environmental samples, animals and poultry.

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.<sup>2,4</sup>

Preston medium may be used as a second enrichment medium in the procedure recommended by ISO 10272-1<sup>5</sup> for the detection of *Campylobacter* spp. in food, especially in the presence of background flora resistant to 3rd generation β-lactams like cefoperazone not inhibited by the first choice medium mCCD agar.

Meat extract and peptone provide nitrogen, carbon and trace elements for microbial growth; sodium chloride maintains the osmotic balance; lysed horse blood neutralizes the trimethoprim antagonists which may be present as residues in the peptones<sup>1</sup>. The antimicrobials of the selective supplement have the following properties: polymyxin is an antibiotic active against Gram-positive bacteria, trimethoprim mainly inhibits the growth and swarming of *Proteus* spp., rifampicin has an inhibitory activity on Gram-positive and Gram-negative bacteria other than *Campylobacter*, cycloheximide is included in the formulation as an antifungal.<sup>1</sup>

### 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20 g in 475 mL of cold purified water. Heat to boiling, stirring constantly, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 25 mL of lysed horse blood (REF 90HLX100) and the content of one vial of Preston Antimicrobial Supplement reconstituted with 2 mL of acetone/sterile distilled water (1:1) under aseptic conditions. Mix well and pour into sterile Petri dishes.



### 5 - PHYSICAL CHARACTERISTICS

#### Campylobacter Blood Agar Base

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.5 ± 0.2

#### Preston Antimicrobial Supplement

Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red, limpid

### 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Agar Base	Dehydrated medium	4012852	500 g (12.5 L)
Preston Antimicrobial Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

### 8 - SPECIMENS

Good laboratory practices for collection, transport and storage of the samples should be applied. For non-clinical samples, refer to the applicable international standard.<sup>5</sup>

### 9 - TEST PROCEDURE

Allow plates to come to room temperature.

**Food chain samples<sup>5</sup>:** detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters, e.g., cooked or frozen products.

- Combine a quantity of 10 g or 10 mL of the test portion with 90 mL of the enrichment medium Bolton broth\* so to obtain a 1 in 10 dilution, and homogenize.
- Incubate the initial suspension in a microaerobic atmosphere at 37 ± 1°C for 4 h to 6 h, then at 41.5 ± 1°C for 44 h ± 4 h.
- Using the culture obtained in the enrichment medium inoculate with a sterile 10 µL loop the surface of the first selective isolation medium, mCCD agar\*\*. Proceed in the same manner with the second *Campylobacter* selective isolation medium chosen by the user (e.g. Preston Medium)\*\*\*
- Incubate the plates at 41.5 ± 1°C in a microaerobic atmosphere for 44 h ± 4 h.
- After 44 h ± 4 h of incubation, examine the plates for typical and/or suspect colonies of *Campylobacter*.

#### Notes

\* Bolton Broth: Campylobacter Bolton Enrichment Broth Base, REF 401286B, added with Bolton Broth Selective Supplement REF 4240025 and Lysed Horse Blood REF 90HLX100.

\*\* mCCD Agar: Campylobacter Free Medium Base (mCCDA), REF 4012822, with the addition of CCDA Antimicrobial Supplement (REF 4240020).

\*\*\* Using a second plating medium with selective agents different from those in mCCD agar could improve *Campylobacter* detection, especially in the presence of background flora resistant to 3<sup>rd</sup> generation β-lactams like cefoperazone.

### 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.

The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat, not only from strain to strain, but also from batch to batch of the selective culture medium used.<sup>5</sup>

*Campylobacter* species are oxidase positive. If a colony phenotypically resembling *Campylobacter* species is oxidase negative, subculture to blood agar and retest after 24h incubation.<sup>6</sup>

The presumptive identification of thermophilic and enteropathogenic *Campylobacter* can be done on the basis oxidase test (+), the absence of growth with an incubation at 25°C and the characteristic microscopic morphology/motility (curved bacilli with a spiralling "corkscrew" motility).

For a complete explanation of the identification criteria and methods, refer to the quoted references.<sup>5,6</sup>

### 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 his 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.<sup>10</sup>

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>C.jejuni</i> ATCC 33291	39-42°C / 40-48h / M	good growth
<i>C.coli</i> ATCC 43478	39-42°C / 40-48h / M	good growth
<i>E.coli</i> ATCC 25922	39-42°C / 40-48h / M	partially or totally inhibited
<i>S.aureus</i> 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 Agar Base, supplemented with Preston Antimicrobial 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; Preston Medium plates are inoculated with decimal dilutions in saline of the colonies suspensions and incubated 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  $\geq 0.7$  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, *P.mirabilis* ATCC 29906. *C.albicans* is partially inhibited, the growth of other non-target strains is totally inhibited<sup>1</sup>.

### 13 - LIMITATIONS OF THE METHOD

- With incubation at 42-43°C some strains of *C.fetus*, *C.upsaliensis* *C.jejuni* subsp.*doylei* may not grow on the medium.<sup>1</sup>
- Campylobacter is sensitive to the moisture level on plating media: dry plates yield smaller colonies, the more humid the medium the more colonies develop with typical morphology.<sup>1</sup>
- If possible, use the plates on the day of their preparation. If necessary, store laboratory prepared plates for no more than 5 days in the dark at 2-8 °C. Excessive drying of the plates can lead to diagnostic errors.<sup>1</sup>
- Prolonged incubations beyond 48 hours may result in the development of contaminants that mask the growth of Campylobacter.<sup>3</sup>
- To achieve the highest yield of *Campylobacter* from stool samples or food, a combination of media that includes Preston medium and a second selective medium, based on a different selective system, appears to be the optimal method (e.g., mCCDA).<sup>5,10</sup>
- 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 and the supplement are for microbiological control only and for professional use; 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](http://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 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 supplements 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](http://www.biolifeitaliana.it).
- Although the intended use and the test procedure of the medium refers to the detection of Campylobacter in food and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of the medium for human clinical specimens. Clinical applications should be validated by the user.
- 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 +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).

#### Selective supplement

Upon receipt, store the product in the original package at +2°C /+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) 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. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. *J Clin Pathol* 1982; 35:462
3. Bolton FJ, Coates D, Hinchliffe PM, Robertson L. Comparison of selective media for isolation of Campylobacter jejuni/coli. *J Clin Pathol* 1983; 36:78-83.
4. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? *J Clin Pathol* 1983; 36:1350-1352
5. ISO 10272-1:2017: Microbiology of the food chain — Horizontal method for detection of Campylobacter spp.
6. Fitzgerald C, Nachamkin I. Campylobacter and Arcobacter. *In* Jorgensen JH, Carroll KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015. p.998.
7. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
8. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018





9. Hunt JM, Abeyta C, Tran T. *In* U.S. Food and Drug Administration Bacteriological Analytical Manual. Chapter 7 *Campylobacter*. January 2001. Content current as of: 03/26/2018.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. 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

### TABLE OF APPLICABLE SYMBOLS

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

### REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/09
Revision 2	Update of "intended use", "test procedure", "precautions and warnings" and "storage conditions and shelf life"	2022/05
Revision 3	Updated content	2023/11

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

