



CAMPYLOBACTER ENRICHMENT BROTH BASE PRESTON ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplement

1 - INTENDED USE

Medium base and selective supplement for the enrichment of *Campylobacter* spp. in food samples.

2 – COMPOSITION*

CAMPYLOBACTER ENRICHMENT BROTH BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10 g
Beef extract	10 g
Sodium chloride	5 g
Agar	1 g

PRESTON ANTIMICROBIC SUPPLEMENT

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim lactate	5 mg
Polymyxin B	2500 IU

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter Enrichment Broth Base with the addition of Preston Antimicrobial Supplement and lysed horse blood, corresponds to the medium proposed by ISTISAN Report 86-95¹ to aid resuscitation of sub-lethally damaged campylobacters. The medium represents a modification of the original formula described by Bolton and Robertson² and recommended by ISO 17995³ as it contains 1 g/L of agar. Peptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Agar, included at a concentration of 0.1 %, aids in initialization of the growth of *Campylobacter* and allows their growth from low inocula; it also retards the dispersion of CO₂, diffusion of oxygen and reducing substances. The selective agents of the medium are polymyxin B, active against Gram-negative bacteria, trimethoprim which mainly suppresses the growth of *Proteus* spp. and other Gram-negative bacteria, rifampicin active against Gram-negative and Gram-positive bacteria and cycloheximide, included as an antifungal compound. Lysed blood horse provides specific and essential nutritional factors for the growth of campylobacters.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 13 g in 500 mL of purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Preston Antimicrobial Supplement (REF 42540017) reconstituted with 2 mL of 50% acetone/sterile purified water, and 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Campylobacter Enrichment Broth Base

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Prepared tubes appearance	dark red, 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 solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Campylobacter Enrichment Broth Base	Dehydrated medium	4012862	500 g (19.2 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, sterile pipettes and loops, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile tubes or flasks, controlled atmosphere generators and jars, lysed horse blood (REF90HLX100), ancillary culture media and reagents.

8 - SPECIMENS

Food samples. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

- In general, for preparing the initial suspension, combine a quantity of 25 g or 25 mL of the test portion with 225 ml of the enrichment medium, so as to obtain a 1 in 10 dilution, and homogenize.
- Incubate the initial suspension in a microaerobic atmosphere at 42 °C for 18 hours.
- Place a pipette about 2 cm below the meniscus of the broth and take an aliquot.
- Sow 3-5 drops on the surface of a plate of Skirrow medium and a second selective medium such as Karmali or CCDA Bolton.
- Streak the entire agar surface to obtain well-isolated colonies and incubate at 42°C for 24-48 hours in a microaerophilic atmosphere.
- Select 5 typical colonies from the selective media and proceed to confirmation tests.



**10 - READING AND INTERPRETATION**

Microbial growth in the selective enrichment broth is evidenced by the development of turbidity.

After incubation of isolation plated media, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

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 and catalase, an aerobic growth test at 25°C, acid and gas in Triple Sugar Iron Agar (TSI), test with cephalothin discs. Optionally, the *Campylobacter* species are identified by specific biochemical tests and/or molecular methods.

The criteria for the identification of thermophilic *Campylobacter* and for the differentiation between species are indicated in the following tables.

Main characteristics of thermophilic *Campylobacter*

Cell morphology	small, slender, curved to spiral, Gram-negative rods
Motility	typical darting motility
Growth at 25°C	negative
Oxidase	positive
Catalase	positive
Acid/gas in TSI	negative
Cephalothin	resistant

Differential tests for *C. jejuni*, *C. coli*, *C. lari*

Test	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>
H ₂ S in TSI	-	-	-
Nalidixic Acid	S	S	R
Hippurate Hydrolysis	+	-	-

S = susceptible R = resistant

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
<i>C. jejuni</i> 33291+ <i>E. coli</i> 25922+ <i>P. mirabilis</i> 29906	42°C 18 h / M	> 10 characteristic colonies on mCCD agar
<i>C. coli</i> 43478+ <i>E. coli</i> 25922+ <i>P. mirabilis</i> 29906	42°C 18 h / M	> 10 characteristic colonies on mCCD agar
<i>E. coli</i> ATCC 25922	42°C 18 h / M	no growth after subculture on Tryptic Soy Agar
<i>P. mirabilis</i> ATCC 29906	42°C 18 h / M	no growth after subculture on Tryptic Soy Agar

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* Enrichment Broth Base, supplemented with Preston Antimicrobial Supplement (REF 4240017) and lysed horse blood, are tested for productivity and selectivity by comparing the results with previously approved Reference Batches.

Productivity and selectivity are assessed also with mixtures of target and non-target strains in the same Preston broth tubes:

- 1) *C. jejuni* ATCC 33291+*E. coli* ATCC 25922+*P. mirabilis* ATCC 29906
- 2) *C. coli* ATCC 43478+*E. coli* ATCC 25922+*P. mirabilis* ATCC 29906.

After incubation of the tubes at 37°C for 18 hours in microaerobic atmosphere and sub-culture onto mCCDA Agar plates, *Campylobacter* strains exhibit a good growth (>10 CFU/plate).

Productivity is tested by a semi-quantitative test with the target strains *C. coli* ATCC 29428 and *C. jejuni* ATCC 33291. Preston broth tubes are inoculated with decimal dilutions in saline of the test-strains and incubated at 42°C for 18 hours in microaerobic atmosphere. After incubation the growth in the enrichment broth is inoculated onto the plates of mCCD Agar. Target strains exhibit a good growth on mCCD Agar (> 10 CFU).

Selectivity is assessed by a semi-quantitative test with non-target strains *E. coli* ATCC 25922 and *P. mirabilis* ATCC 29906. Preston broth tubes are inoculated according to ISO 11133⁴ and incubated at 37°C for 18 hours in microaerobic atmosphere. After incubation the growth in the enrichment broth is inoculated onto Tryptic Soy Agar plates. The non-target strains do not exhibit growth on Tryptic Soy Agar.

13 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement 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. Preston Antimicrobial 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 the supplement vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- 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 medium and supplement and the inoculated tubes/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.

14 - 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).

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 (tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 17995³ the complete Preston broth should be stored at 5 ± 3°C for not more than 7 days.

15 - REFERENCES

1. Istituto Superiore di Sanità -Metodi di analisi per il controllo microbiologico degli alimenti – Raccolta a cura di D.De Medici, L.Feniccia, L.Orefice, A.Stacchini 1996, iv, 166 p. Rapporti ISTISAN 96/35
2. Bolton FJ, Robertson L. A selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol 1982 Apr; 35(4):462-7.
3. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant *Campylobacter* spp
4. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

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	2022/11

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

