

NUTRIENT BROTH N° 2 PRESTON ANTIMICROBIC SUPPLEMENTS CAMPYLOBACTER GROWTH SUPPLEMENT (Preston broth)

Dehydrated culture medium and supplements

1 - INTENDED USE

General purpose liquid medium the cultivation of fastidious and non-fastidious microorganisms; medium base for the preparation of Preston broth for the enrichment of *Campylobacter* spp. in food and water samples.

2 – COMPOSITION*

DEHYDRATED NUTRIENT BROTH N° 2 (REF 401812)

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of animal tissues	10 g
Peptone	10 g
Sodium chloride	5 g

PRESTON ANTIMICROBIC SUPPLEMENT II (REF 4240022)

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B	2500 IU
Amphotericin B	5 mg
Rifampicin	5 mg
Trimethoprim	5 mg

PRESTON ANTIMICROBIC SUPPLEMENT (REF 4240017)

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

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

CAMPYLOBACTER GROWTH SUPPLEMENT (REF 4240021)

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Sodium pyruvate	125 mg
Sodium metabisulfite	125 mg
Ferrous sulphate	125 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Preston broth, prepared with Nutrient Broth n° 2 and the addition of Preston Antimicrobial Supplement, Campylobacter Growth Supplement and lysed horse blood, corresponds to the medium proposed by Bolton *et al.* in 1982^{1,2} for estimating small numbers of campylobacters in water.

The current method described by ISO 10272-2³ for food chain samples, recommends Bolton Broth as well as Preston broth, with or without Campylobacter Growth Supplement and with cycloheximide substituted by amphotericin B. Preston broth is recommended as a selective enrichment medium for samples with low numbers of campylobacters and high level of background microflora (ISO detection procedure B). There is a similar ISO method for water (ISO17995)⁴ which uses Preston broth (with Campylobacter Growth Supplement) as well as Bolton broth.

The addition of growth supplement to Preston Broth enhances recovery of *Campylobacter* spp. and some strains will not grow without it.³ The use of Preston broth overcomes problems with background flora resistant to third generation β -lactams, like cefoperazone in Bolton broth.

Beef extract and peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Sodium pyruvate aids in resuscitation of stressed cells and, together with sodium metabisulfite and sodium sulphate, enhances the isolation and the oxygen tolerance of *Campylobacter* spp.⁵ Lysed blood horse provides specific and essential nutritional factors for the growth of campylobacters. 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 amphotericin B, included as an antifungal compound.

Nutrient Broth N°2 without the addition of supplements may be used as a general-purpose liquid medium for the cultivation of fastidious and non-fastidious microorganisms. It gives good growth from small inocula and is particularly suitable for subculture of staphylococci for the coagulase test.

4- DIRECTIONS FOR MEDIUM PREPARATION

General purpose medium

Suspend 25 g in 1000 mL of purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

Preston broth

Suspend 25 g in 945 ml of purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Sterilize by autoclaving at 121°C for 15 minutes.

Cool to 47-50°C and add 50 ml of lysed horse blood (REF 90HLX100) and the contents of two vials of Preston Antimicrobial Supplement II (REF 4240022 with amphotericin B) or Preston Antimicrobial Supplement (REF 4240017 with cycloheximide) reconstituted with 2 mL of





50% acetone/sterile distilled water. If required add also the contents of 2 vials of *Campylobacter* Growth Supplement (REF 4240021), reconstituted with 5 mL of sterile purified water. Mix well and distribute into sterile tubes or flasks.

5 - PHYSICAL CHARACTERISTICS

Nutrient Broth n°2

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	7.4 ± 0.2

Preston Antimicrobial Supplement (with cycloheximide)

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

Preston Antimicrobial Supplement II (with amphotericin B)

Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red-orange opalescent solution

Campylobacter Growth Supplement

Freeze-dried supplement appearance	short, friable, green-grey pastille
Reconstituted supplement appearance	grey-green limpid solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Broth n° 2	Dehydrated medium	4018122	500 g (20 L)
Preston Antimicrobial Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium
Preston Antimicrobial Supplement II	Freeze-dried supplement	4240022	10 vials, each for 500 mL of medium
<i>Campylobacter</i> Growth Supplement	Freeze-dried supplement	4240021	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, Erlenmeyer flasks, sterile tubes, controlled atmosphere generators and jars, lysed horse blood, 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^{3,4} for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

Food samples

1. According to ISO 10272-1, Preston broth is used as selective enrichment broth for the detection of *Campylobacter* in samples with low numbers of campylobacters and high level of background microflora, e.g., raw meats (including poultry) or raw milk.
2. In general, for preparing the initial suspension, combine a quantity of 10 g or 10 mL of the test portion with 90 ml of the enrichment medium (Preston broth), so as to obtain a 1 in 10 dilution, and homogenize.
3. Incubate the initial suspension in a microaerobic atmosphere at 41.5 ± 1 °C for 24 h ± 2 h.
4. Using the culture obtained in the enrichment medium, inoculate with a sterile 10 µl loop the surface of the isolation medium, mCCDA Agar*.
5. Incubate the selective solid medium at 41.5 °C in a microaerobic atmosphere and examine after 44 h to detect the presence of typical and/or suspect *Campylobacter* colonies.

Water samples

1. In general, the detection of *Campylobacter* in water according to ISO 17995⁴ requires enrichment followed by isolation of colonies and their confirmation.
2. Samples are inoculated either directly or after concentration using membrane filtration into one of two selective enrichment broths depending on the expected level of background microorganisms: Bolton broth[^] for clean water and Preston broth for more heavily contaminated water. A single sample volume is processed for *Campylobacter* detection and, where necessary, at least three 10-fold volumes (for example 10 mL, 100 mL and 1000 mL) are used for a semi-quantitative determination. For a quantitative (MPN) determination, volumes of 500 mL, 5 x 100 mL, 5 x 10 mL and, where counts may be high, smaller volumes are used or the initial sample is diluted. The broths are then incubated microaerobically at 37 ± 1 °C for 44 ± 4 h.
3. From the enrichment broth cultures, liquid selective media are inoculated onto mCCDA agar* and the plates are incubated at 41.5 ± 1 °C for 44 ± 4 h in a microaerobic atmosphere.

Notes

*mCCDA agar: *Campylobacter* Blood Free Medium Base Bolton REF 401282 + Bolton CCDA Antimicrobial Supplement REF 4240020

[^]Bolton broth: *Campylobacter* Bolton Broth Base REF 401286B2 + Bolton Broth Selective Supplement REF 4240025.

10 - READING AND INTERPRETATION

Microbial growth in Preston 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.

Campylobacter colonies usually are greyish on mCCDA 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.

Characteristics of *Campylobacter*:

morphology: small curved bacilli, motility: characteristic corkscrew darting, aerobic growth at 25°C: negative, oxidase test: positive

C. coli, *C. jejuni*, *C. lari*, *C. upsaliensis* can be differentiated by catalase test, hydrolysis of hippurate and indoxyl acetate test

As an alternative, or in addition, to the confirmation and identification tests, other tests such as PCR test, serological methods, MALDI-TOF-MS analysis, can be used.

For a complete explanation of the identification criteria and methods, refer to the quoted references.^{3,4}





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. Depending on the intended use (e.g., detection of *Campylobacter* in water or foodstuffs), apply the quality control required by the Standards.^{3,4,8}

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated Nutrient Broth n° 2, 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 tested with mixtures of target and non-target strains in the same Preston broth tubes:

- 1) *C. jejuni* ATCC 29248+*E. coli* ATCC 25922+*S. aureus* ATCC 6538.
- 2) *C. coli* ATCC 43478+*E. coli* ATCC 25922+ *S. aureus* ATCC 6538.

After incubation of the tubes at 41.5°C for 44 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 33291. Preston broth tubes are inoculated with decimal dilutions in saline of the test-strains and incubated at 41.5°C for 24 ± 2 hours in microaerobic atmosphere. After incubation the growth on the enrichment broth is inoculated onto the plates of mCCDA Agar. Target strains exhibit a good growth on mCCDA Agar (> 10 CFU).

Selectivity is assessed by a semi-quantitative test with non-target strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Preston broth tubes are inoculated at 41.5°C for 24 ± 2 hours in microaerobic atmosphere. After incubation the growth on the enrichment broth is inoculated onto Tryptic Soy Agar plates. The non-target strains do not exhibit growth on Tryptic Soy Agar.

13 - LIMITATIONS OF THE METHOD

- The procedures described above target the thermotolerant *Campylobacter* spp. relevant for human health. The most frequently encountered strains are *C. jejuni* and *C. coli*. However, other species have been described (*C. lari*, *C. upsalinesis* and others).³
- The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat.³

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements 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 supplements 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. Preston Antimicrobial Supplement and Preston Antimicrobial Supplement II are classified as hazardous. 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 supplements vials to avoid injury.
- The supplements are 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 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 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

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 supplements

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 lyophilized 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 10272³ and ISO 17995⁴ the complete Preston broth should be stored at 5 ± 3°C for not more than 7 days.

16 - REFERENCES













1. Bolton FJ, Hinchliffe PM, Coates D, Robertson L. A most probable number method for estimating small numbers of campylobacters in water. J Hyg (Lond). 1982 Oct; 89(2):185-90.
2. Bolton FJ, Robertson L. A selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol 1982; 35(4):462-7.





3. ISO 10272-1:2017+ A1: 2023. Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Detection method.
4. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant *Campylobacter* spp.
5. Hoffman PS, George HA, Krieg NR, Smibert RM. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. jejuni. II. Role of exogenous superoxide anions and hydrogen peroxide. *Can J Microbiol* 1979 Jan; 25(1):8-16.

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	2023/02

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

