



## Bolton broth

# CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE

# BOLTON BROTH SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplement

**1 - INTENDED USE**

Selective liquid medium for the enrichment of *Campylobacter* spp. in food and water samples.

**2 – COMPOSITION\*****CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE****TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)**

Enzymatic digest of animal tissue	10.00 g
Lactalbumin hydrolysate	5.00 g
Yeast extract	5.00 g
Sodium chloride	5.00 g
Sodium pyruvate	0.50 g
Sodium metabisulfite	0.50 g
Sodium carbonate	0.60 g
α- ketoglutaric acid	1.00 g
Haemin	0.01 g

**BOLTON BROTH SELECTIVE SUPPLEMENT****(VIAL CONTENTS FOR 500 ML OF MEDIUM)**

Cefoperazone	10 mg
Vancomycin	10 mg
Trimethoprim lactate	10 mg
Amphotericin B	5 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**

Bolton broth, prepared with Campylobacter Bolton Enrichment Broth Base and the addition of Bolton Broth Selective Supplement and lysed horse blood, corresponds to the medium proposed by Bolton<sup>1</sup> to aid resuscitation of sub-lethally damaged campylobacters.

The current ISO method<sup>2</sup> for foods in general recommends the use of Bolton broth as a selective enrichment medium for samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters. There is a similar ISO method for water which uses Bolton broth as well as Preston broth.<sup>3</sup> The selective enrichment in Bolton Broth is also recommended by FDA-BAM.<sup>4</sup>

The enzymatic digest of animal tissue and lactalbumin hydrolysate provide nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly the B-group. Sodium pyruvate aids in resuscitation of stressed cells and with sodium metabisulfite and sodium carbonate enhances the isolation and the oxygen tolerance of *Campylobacter* spp. Sodium chloride maintains the osmotic balance. Alpha-ketoglutarate, hemin and lysed horse blood provide specific and essential nutritional factors for the growth of campylobacters. The selective agents of the medium are vancomycin active against Gram-positive bacteria, trimethoprim and 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 13.8 g in 470 mL of purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool below 47 °C and add the contents of one vial of Bolton Broth Selective Supplement (REF 4240025) reconstituted with 5 mL of 50% ethanol/sterile distilled water, together with 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks.

**5 - PHYSICAL CHARACTERISTICS****Campylobacter Bolton Enrichment Broth Base**

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow limpid
Prepared tubes appearance	dark red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

**Bolton Broth Selective Supplement**

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

**6 - MATERIALS PROVIDED - PACKAGING**

Product	Type	REF	Pack
Campylobacter Bolton Enrichment Broth Base	Dehydrated medium	401286B2	500 g (18.1 L)
Bolton Broth Selective Supplement	Freeze-dried supplement	4240025	10 vials, each for 500 mL of medium

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Autoclave, water-bath, sterile loops and pipettes, 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<sup>2-4</sup> for the collection, transport, storage of samples and operate in accordance with good laboratory practice.





## 9 - TEST PROCEDURE

### Food samples<sup>2</sup>

According to ISO 10272-1, Bolton broth is used as selective enrichment broth for detection of *Campylobacter* in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters.

In general, for preparing the initial suspension, combine a quantity of 10 g or 10 mL of the test portion with 90 mL of Bolton broth, so as to obtain a 1 in 10 dilution, and homogenize.

Incubate in a microaerobic atmosphere at 37°C ± 1°C for 4 h to 6 h, then at 41.5°C ± 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 two selective isolation media: mCCD agar\* and a second *Campylobacter* selective isolation medium using a different selective principle.

Incubate the selective solid media at 41.5°C ± 1°C in a microaerobic atmosphere and examine after 44 ± 4 h to detect the presence of suspect *Campylobacter* colonies.

### Water samples<sup>3</sup>

In general, the detection of *Campylobacter* in water according to ISO 17995 requires enrichment followed by isolation of colonies and their confirmation.

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<sup>4</sup> 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.

From the enrichment broth cultures, liquid selective media are inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA)\*. The mCCDA plates are then 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 42400120

<sup>4</sup>Preston broth: Nutrient Broth n° 2 REF 401812S2 + Preston Antimicrobial Supplement REF 4240022 + Lysed Horse Blood REF 90HLX100.

## 10 - READING AND INTERPRETATION

Microbial growth in Bolton 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 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 references.<sup>2-4</sup>

## 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 according to ISO 10272:

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS (AFTER SUB-CULTURE)
<i>C. jejuni</i> 33291+ <i>E. coli</i> 25922+ <i>P. mirabilis</i> 29906	37±1°C / 44±4 h / M	> 10 characteristic colonies on mCCD agar
<i>C. coli</i> 43478+ <i>E. coli</i> 25922+ <i>P. mirabilis</i> 29906	37±1°C / 44±4 h / M	> 10 characteristic colonies on mCCD agar
<i>E. coli</i> ATCC 25922	37±1°C / 44±4 h / M	no growth after subculture on Tryptic Soy Agar
<i>P. mirabilis</i> ATCC 29906	37±1°C / 44±4 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* Bolton Enrichment Broth Base supplemented with Bolton Broth Selective Supplement, are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity and selectivity are tested with mixtures of target and non-target strains in the same Bolton 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 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 29428 and *C. jejuni* ATCC 33291. Bolton broth tubes are inoculated with decimal dilutions in saline of the test-strains and incubated at 37°C for 44 hours in microaerobic atmosphere. After incubation the growth on 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. Bolton broth tubes are inoculated according to ISO 11133<sup>5</sup> and incubated at 37°C for 44 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 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are intended 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 Broth Selective 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](http://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 supplement 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](http://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 +2°C /+8°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 10272 the complete Bolton broth should be stored at 5 ± 3°C for not more than 7 days.<sup>2</sup> According to ISO 17995, the basal broth should be stored at 5 ± 3°C for not more than six months in the dark in airtight bottles.<sup>3</sup>

### 15 - REFERENCES

1. Bolton, F. J. Methods for the isolation of campylobacters from humans, food and water. In: The Increasing Incidence of Human Campylobacteriosis. Report and Proceedings of a WHO Consultation of Experts, Copenhagen, Denmark, 21-25 November 2000, pp. 87 – 93
2. ISO 10272-1:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method
3. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp
4. FDA-BAM Chapter 7: Campylobacter. Content current as of: 08/03/2021
5. 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 2	Updated layout and content	2022/06

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

