

## Bolton broth base (ISO)

Code 84697.0500

### Intended use

Liquid medium base for the enrichment of *Campylobacter* spp. in food and animal feeding stuffs.

### Formula\* - Composition in g/L

Enzymatic digest of animal tissues.....	10.00
Lactalbumin hydrolysate.....	5.00
Yeast extract.....	5.00
Sodium chloride.....	5.00
Sodium pyruvate.....	0.50
Sodium metabisulphite.....	0.50
Sodium carbonate.....	0.60
$\alpha$ -ketoglutaric acid.....	1.00
Haemin.....	0.01

\* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.4  $\pm$  0.2 at 25 °C.

### Instructions for preparation

Dissolve 13.8 g in 500 ml of purified water by heating if necessary. Sterilise in the autoclave at 121 °C for 15 minutes and cool to 44-47 °C. Dissolve the contents of one vial of Bolton broth selective supplement (Art.: 84744.0001) with of 50% ethanol/sterile distilled water. To the medium base add 25 ml of lysed horse blood, then the reconstituted supplement. Mix well and distribute into sterile tubes or flasks.

### Principle of the method and general information

Bolton broth base, prepared according to the formulation proposed by ISO 10272-1, is used in the enrichment procedure for the detection of *Campylobacter* spp. growing at 41,5°C.

Enzymatic digest of animal tissue, lactalbumin hydrolysate and yeast extract supply nitrogen, vitamins, minerals, and amino acids needed for the bacterial growth. Sodium chloride is used to maintain the osmotic equilibrium and sodium carbonate to maintain the pH around neutral values.

Sodium metabisulphite and sodium pyruvate reduce toxic compounds and increase the recovery rate and aero-tolerance of the cultures. Alpha-ketoglutaric acid stimulate the bacterial metabolism. The antimicrobials are supplied by the freeze-dried selective supplement containing vancomycin, cefoperazone and trimethoprim for the inhibition of Gram-positive and Gram-negative bacteria and Amphotericin B as an antifungal agent.

### Instruction for use

For laboratory use only.

For the detection of *Campylobacter* in food and animal feeding stuffs, ISO 10272 recommends the method described below.

- For preparing the initial suspension, introduce a quantity x (g or ml - mass or volume) of the test portion into nine times its volume of Bolton broth so as to obtain a test portion/enrichment medium ratio of 1:10 and homogenize.
- Incubate the initial suspension in a micro-aerophilic atmosphere at 37 °C for 4 h to 6 h, then at 41.5°C for 44 h  $\pm$  4 h.
- Using the culture obtained in the enrichment medium, inoculate with a sterile loop the surface of the first selective medium: Modified charcoal cefoperazone (CCD) agar, (Art. N° 84695-0500).
- Proceed in the same manner for the second isolation medium (e.g. Karmali medium base, Art. N° 84696-0500)
- Incubate the plates at 41,5°C for 44 h  $\pm$  4 h in a microaerophilic atmosphere
- After 44 h  $\pm$  4 h of incubation, examine the plates for typical and/or suspected colonies of *Campylobacter*. The typical colonies on CCD agar and Karmali agar are greyish often with a metallic

sheen and are flat and moist with a tendency to spread. Colonies spread less on dried agar surfaces. Other forms of colonies may occur.

- For confirmation tests, take from each plate of selective medium at least one colony considered to be typical or suspected and a further four colonies if the first is negative. Streak a plate of Columbia blood agar in order to allow the development of well isolate colonies. Incubate the plates at 41,5°C for 24-48 h in a micro-aerophilic atmosphere and perform the confirmatory tests and, if required, the identification tests.

### Limitations

- It is recommended that biochemical and/or serological tests be performed on pure culture for complete identification.

### Quality Control

Physical characteristics:

Appearance of powder	Beige, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Yellow, limpid
pH (25°C)	7.4 ± 0.2

Microbiological characteristics:

Test Strains	Incubation T° / t / At.	Inoculation method	Growth characteristics
<i>C. coli</i> ATCC 42478	ISO/TS	ISO/TS	Good growth, presence of more than 10 colonies onto Bolton CCDA plates
<i>C. jejuni</i> ATCC 33291	ISO/TS	ISO/TS	Good growth, presence of more than 10 colonies onto Bolton CCDA plates
<i>E. coli</i> ATCC 25922	ISO/TS	ISO/TS	No growth onto TSA plates
<i>P.mirabilis</i> ATCC 29906	ISO/TS	ISO/TS	No growth onto TSA plates
Mixture of 0.01 ml of appropriate dilution of test strains according to ISO/TS 11133-2			
<i>C. jejuni</i> ATCC 33291	ISO/TS	M3	>10 colonies on the subculture on CCDA agar plate
<i>E. coli</i> ATCC 25922	ISO/TS	M3	
<i>P.mirabilis</i> ATCC 29906	ISO/TS	M3	

### Notes

Medium supplementation: Bolton Broth Selective Supplement (REF 84744.0001) and 25 ml/L of lysed horse blood  
 Inoculation method ISO/TS: 0.01 ml of appropriate dilution of test strains according to ISO/TS 11133-2, subculture onto CCDA agar plate  
 Inoculation method M3: mixture of 0.01 ml of appropriate dilution of test strains acc. to ISO/TS 11133, subculture onto CCDA agar plate  
 Incubation method ISO/TS: 5 hours at 37° than 44 hours at 41,5°C  
 Incubation atmosphere microaerophilic incubation  
 Microbiological characteristics tested in accordance to ISO/TS 11133-2  
 ATCC is a registered trade mark of American Type Culture Collection

### References

- ISO 10272-1:2006 - Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 1: Detection method.

### Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10°C to 30°C and <60% RH).

### Ordering information

Dehydrated medium:

84697.0500 Bolton broth base (ISO) Bottle of 500 g

Supplement:

84744.0001 Bolton broth selective supplement 10 vials, each for 500 ml of complete medium