

**CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE****BOLTON BROTH SELECTIVE SUPPLEMENT**

**Powdered medium and selective supplement for the enrichment  
of *Campylobacter* spp. in foods and animal feeding stuffs acc. to ISO 10272**

**TYPICAL FORMULAS****CAMPYLOBACTER ENRICHMENT BROTH BASE (g/l)**

Enzymatic Digest of Animal Tissue	10
Lactalbumin Hydrolysate	5
Yeast Extract	5
Sodium Chloride	5
Sodium Pyruvate	0,5
Sodium Metabisulfite	0,5
Sodium Carbonate	0,6
$\alpha$ - Ketoglutaric Acid	1
Haemin	0,01

**BOLTON BROTH SELECTIVE SUPPLEMENT (per vial)**

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

**DIRECTIONS FOR POWDERED MEDIUM**

Suspend 13,8 g in 500 ml of distilled water; heat to boiling with agitation, autoclave at 121°C for 15 minutes. Cool to 44-47°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.

**DESCRIPTION**

Campylobacter Enrichment Broth Bolton, prepared according to the formulation given by ISO 10272-1, is used for the enrichment procedure for the detection of *Campylobacter* growing at 41,5°C.

**TECHNIQUE**

- For preparing the initial suspension, introduce a quantity  $x$  of the test portion (mass or volume) into nine times its volume of Campylobacter Broth Bolton so as to obtain a test portion/enrichment medium ratio of 1:10 and homogenize.
- Incubate the initial suspension in a microaerophilic 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 (Campylobacter CCDA Bolton Agar, REF 401282).
- Proceed in the same manner for the second isolation medium, chosen between Skirrow Agar or Karmali Agar or Preston Agar.
- 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 Campylobacter CCDA Bolton 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 microaerophilic atmosphere and perform the confirmatory test (see table 1) and, if required, the identification tests (see table 2)

**TABLE 1 – Characteristics of *Campylobacter* spp**

Morphology	Small curved gram negative bacilli
Motility	characteristic
Growth at 25 °C in a microaerophilic atmosphere	negative
Growth at 41.5 °C in aerobic atmosphere	negative
Oxidase	positive

**TABLE 2 – Characterisation of *Campylobacter* species**

Test	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. upsaliensis</i>
Catalase	+	+	+	- or slight +
Nalidixic Acid	S	S	R/S	S
Cephalotin	R	R	R	S
Hydrolysis of hippurate	+	-	-	-
Indoxyl acetate	+	+	-	+

S = sensitive R = resistant

**User quality assurance** (48 h-42 °C, reduced O<sub>2</sub>)Positive control: *C.jejuni* ATCC 33291: growth**Storage**

Dehydrated medium: 2 - 8 °C

Selective Supplement: 2 - 8 °C

**Packaging**

Powdered medium

**401286B2 *Campylobacter* Bolton Enrichment Broth Base, 500 g (18,1 L)**

Supplements

**4240025 Bolton Broth Selective Supplement****10 vials, each for 500 ml of medium****90MLX100 Lysed horse blood****100 ml****REFERENCE**

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