

Bolton Selective Enrichment Broth Base ISO

Cat. 1441

For the selective preenrichment of *Campylobacter* in foods samples.

Practical information

Applications	Categories
Selective enrichment	Campylobacter

Industry: Food

Regulations: ISO 10272 / ISO 11133 / BAM

Principles and uses

Bolton Selective Enrichment Broth is used for the selective preenrichment of *Campylobacter* in food samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters. It is recommended by ISO 10272.

Campylobacter genus are gram-negative, microaerophilic bacteria that can be present in milk, none treated water or undercooked food.

Injured organisms are not generally detected and therefore a recovery step must be included in examination procedures. This is of importance, particularly in the food industry as various processes such as heat, desiccation, preservation processes, pH changes, etc, cause sublethal injuries to *Campylobacter*. The broth is rich in nutrients and produces high resuscitation rates for sublethally injured bacteria and intense growth.

Meat Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactalbumin Hydrolysate provides nitrogen, amino acids, vitamins, and carbon and is specially indicated for culture media for *Campylobacter* growth. Yeast extract is source of vitamins, particularly the B-group. Sodium chloride supplies essential electrolytes for transport. Alpha-ketoglutarate acid is incorporated to satisfy the specific nutritional requirements of *Campylobacter* species. Sodium pyruvate is a source of energy for bacterial metabolism and aids in resuscitation of stressed organisms. Sodium carbonate is a pH regulator. Hemin provides X factor, which stimulates the growth of many microorganisms. The Addition of Bolton Broth Selective Supplement inhibits the accompanying gram-positive bacteria due to Trimetropin, gram-negative bacteria due to the Vancomycin, Cephoperazone and Trimetropin as well as yeasts and moulds due to Cycloheximide.

Formula in g/L

Alpha-ketoglutaric	1	Hemin	0,01
Sodium carbonate	0,6	Sodium chloride	5
Sodium metabisulfite	0,5	Sodium pyruvate	0,5
Yeast extract	5	Enzymatic digest of animal tissues	10
Lactalbumin hydrolysate	5		

Preparation

Suspend 13,8 grams of the medium in 475 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to <47 °C and aseptically add one vial of the Bolton Selective Supplement (Cat. 6070) and 25 ml of sterile lysed horse blood. Homogenize gently and dispense into tubes.

Instructions for use

For the detection and enumeration of *Campylobacter* spp. for samples with low numbers of campylobacters and low level of background microflora and/or stressed campylobacters according to ISO 10272:

- Add the test portion to the liquid enrichment medium Bolton Broth. 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 Bolton broth.
- Incubate in a microaerobic atmosphere at 37 °C for 4 to 6 h, then at 41,5 °C for 44±4 h.
- From the enrichment culture obtained, inoculate two selective media, CCDA agar (Cat. 1129) and any other selective medium using different selective principles.
- Incubate the selective solid media at 41,5 °C in a microaerobic atmosphere for 44 h to detect the presence of suspect *Campylobacter* colonies.
- Examine the suspect *Campylobacter* colonies for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and the confirm by detection of oxidase activity and an aerobic growth test at 25 °C.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Dark amber. With blood: cherry red	7,4 ± 0,2

Microbiological test

According to ISO 11133:

Incubation conditions: (37±1 °C / 5±1 h, then 41,5±1 °C /44±4 h, microaerobic atmosphere).

Inoculation conditions: Target microorganisms (<100 CFU) / Non-target microorganism (>1000 CFU) / Selectivity (10⁴-10⁶ CFU).

Microorganisms	Specification	Characteristic reaction
Proteus mirabilis ATCC 29906	Total inhibition (0) on TSA	
Campylobacter jejuni ATCC 29428 + Escherichia coli ATCC 25922 + Proteus mirabilis ATCC 29906	> 10 characteristic colonies on CCDA	Greyish, flat and moist, sometimes with metallic sheen
Campylobacter coli ATCC 43478 + Escherichia coli ATCC 25922 + Proteus mirabilis ATCC 29906	> 10 characteristic colonies in CCDA	Greyish, flat and moist, sometimes with metallic sheen
Escherichia coli ATCC 8739	Total inhibition (0) on TSA	

Storage

Temp. Min.:2 °C

Temp. Max.:25 °C

Bibliography

ISO 10272-1:2017 Microbiology of the food chain. Horizontal method for detection and enumeration of Campylobacter spp. . Part 1: Detection method

Post, D. E. (1995). Food-Borne Pathogens Monograph Number 3 Campylobacter.

Bolton, F.J. (1995) Personal communication.

Hunt, J.M. (1998) Campylobacter. In: F.D.A. Bacteriological Analytical Manual, 8th Edition (Revision A) 7.01-7.27. AOAC, Arlington Va.