

Specification

A selective supplement for the selective preenrichment of *Campylobacter* in foods samples.

Presentation

10 Freeze dried vials
Vial
with: 6 ± 0.1 g

Packaging Details

$22 \pm 0.25 \times 55 \pm 0.5$ mm glass vials, tag labelled, White plastic cap - 10 vials per box.

Shelf Life

36 months

Storage

2-8 °C

Composition

Compositon (g/vial)

Vancomycin.....0.010
Trimethoprim..... 0.010
Cefoperazone.....0.010
Amphotericin B.....0.005

NOTE: Each vial is sufficient to supplemented
500 ml of Bolton Selective Enrichment Broth.

Reconstitute the original freeze-dried vial

by adding :

Sterile Distilled Water/ Ethanol(50:50)..... 2,5 ml

Description /Technique

Description:

Bolton Selective Enrichment Broth (Cat. 1441) 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 *Campylobacter*. 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 Amphotericin B.

Technique:

Aseptically reconstitute 1 vial with 2,5 ml of sterile distilled water/ethanol 1:1. Mix gently until complete dissolution. Aseptically add one vial to 475 ml of Bolton Selective Enrichment Broth Base (Cat. 1441) autoclaved and cooled to below 47 °C + 25 ml of sterile lysed horse blood. Mix well and distribute into sterile containers.

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

Physical/Chemical control

Color : White-Gray pH: at 25°C

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Microaerophilia. 37°C ± 1 during 5h±1; After 41,5°C±1 during ± 44h ±4

Subculture after incubation onto appropriate media

Microbiological control accor. to ISO 11133:2014/A1:2018.

Microorganism

Campylobacter jejuni ATCC® 29428, WDCM 00156

Escherichia coli ATCC® 25922, WDCM 00013

Proteus mirabilis ATCC® 29906

Campylobacter coli ATCC® 43478

Growth

Good to excelent - Typical colonial appearance

Inhibited

Inhibited

Good to excelent - Typical colonial appearance

Sterility Control

Add 5ml of the sample to 100 ml of TSB and to 100 ml Thioalglycollate.

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

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.