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Bolton Broth Base

Bolton Broth Base is a selective enrichment medium used for the isolation and recovery of Campylobacterspecies from food and animal feed samples, as well as thermotolerant Campylobacter spp. from water samples

REF: BS.1/NZ01.100.0100	100 Gram	REF: BS.1/NZ01.250.0250	250 Gram
REF: BS.1/NZ01.500.0500	500 Gram		

PRINCIPLE AND INTERPERTATION :

Campylobacter species are recognized as important enteric pathogens causing gastrointestinal illness that ranges from mild diarrhoea to severe disease characterized by watery stools that may progress to bloody diarrhoea. Among the species, C. jejuni, C. coli, and C. lari are most frequently associated with human infections. Food products of animal origin constitute the principal source of transmission to humans, with unpasteurized milk being one of the most commonly implicated vehicles in foodborne outbreaks of Campylobacter jejuni enteritis (1,2).Historically, Campylobacter organisms were classified within the genus Vibrio; however, they were later distinguished based on differences in DNA base composition and their ability to grow under reduced oxygen conditions. Early selective media developed for the isolation of C. jejuni from faecal specimens employed a combination of antibiotics incorporated into a nutritionally rich basal medium (3). Bolton Broth Base, originally developed by Bolton (8), is prepared in accordance with the recommendations of ISO and FDA BAM for the detection and enumeration of Campylobacter spp. from food samples, environmental samples collected from food production areas, and water samples (4 – 7). The media is made selective for Campylobacters by addition of the antibiotics cefoperazone, vancomycin, trimethoprim and amphotericin B. These antibiotics are added as freeze dried supplements .The medium contains Peptone, Lactalbumin hydrolysate and yeast extract which aid resuscitation of sub lethally damaged cells of Campylobacter. The supplement added to the medium contains four different antibiotics, vancomycin, cefoperazone and trimethoprim inhibit the growth of grampositive and gram-negative bacteria while amphotericin B largely reduces the growth of yeasts and moulds.

MEDIA COMPOSITION

Item	Formula per liter of medium
Peptone	10 gm
Lactalbumin hydrolysate	5.0 gm
Yeast extract	5.0 gm
Sodium chloride	5.0 gm
Sodium metabisulphite	0.5 gm
Sodium carbonate	0.6 gm
Hemin	0.01 gm
α-ketoglutaric acid	1.0 gm
Sodium pyruvate	0.5 gm

Final pH (at 25°C) 7.4 ±0.2

** Formula adjusted, standardized to suit performance parameters.

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Bolton Broth Base material safety data sheet.

STORAGE AND STABILITY

BioScien **Bolton Broth Base Store** between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

PREPARATION

Suspend 13.80 gram in 500 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Bolton Selective supplement (FD231) and 25 ml of sterile lysed defibrinated horse blood in the medium. Horse blood may be saponin lysed or lysed by freezing then thawing out. Mix well and aseptically dispense into sterile tubes or flasks as desired

Deterioration

The color of ***BioScien*** **Bolton Broth Base** Light yellow to brownish yellow homogeneous free flowing powder. Basal medium : Brownish yellow coloured clear to slightly opalescent solution. After addition of lysed horse blood: Red to brown coloured opaque solution in tubes.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes

- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed with added Bolton Selective Supplement (FD231) after an incubation at 37 ± 1°C for 5± 1 hours and then at 41.5 ± 1°C for 44 hours ± 4 hours in a microaerobic atmosphere. Further subculture is carried out on mCCD Agar at 41.5°C in a micro-aerobic atmosphere for 44 hours ± 4 hours. Selectivity : Cultural characteristics observed with added Bolton Selective Supplement (FD231) after an incubation at 37 ± 1°C for 5± 1 hours and then at 41.5 ± 1°C for 44 hours ± 4 hours in a microaerobic atmosphere. Further subculture is carried out on mCCD Agar at 41.5°C in a micro-aerobic atmosphere for 44 hours ± 4 hours.

Organisms (ATCC)	Growth on M887I - mCCD Agar
Escherichia coli ATCC 25922	Negative reaction
Campylobacter jejuni ATCC 33291	greyish, flat and moist colonies, may have metallic sheen inhibited
Campylobacter jejuni ATCC 29428	greyish, flat and moist colonies, may have metallic sheen
Campylobacter coli ATCC 43478	Positive reaction, blackening of medium
Escherichia coli ATCC 25922	inhibited
Proteus mirabilis ATCC 29906	inhibited

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact ***BioScien*** technical support.

REFERENCE

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4. Hunt J.M, Campylobacter, F.D.A Bacteriological Analytical Manual, 8th Edition (Revision AOAC, Arlington V A (1998).

5.International Organization for Standardization (ISO), 10272-1:2017, Microbiology of the food chain —Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method

6. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, EN ISO 11133:2014 (E) /Amd. : 2020.

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10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		