Technical Data

Bolton Broth Base

M1592

Intended use

Bolton Broth Base is selective enrichment of *Campylobacter* species from food, animal feeding stuffs and thermotolerant *Campylobacter* spp. from water. The composition and performance criteria of this medium are as per the specifications laid down in ISO 10272-1:2017 , ISO 17995:2019(E) and 11133:2014 (E) /Amd. : 2020.

Composition**

ISO Specification - Bolton Broth

Ingredients	g / L
Enzymatic digest of animal tissues	10.000
Lactalbumin hydrolysate	5.000
Yeast extract	5.000
Sodium chloride	5.000
Sodium metabisulphite	0.500
Sodium carbonate anhydrous	0.600
Hemin	0.010
α-ketoglutaric acid, monopotassium salt	1.000
Sodium pyruvate	0.500

Supplements to be added after autoclaving

Cefoperazone sodium salt	0.02g
Vancomycin hydrochloride	0.02g
Trimethoprim lactate salt	0.02g
Amphotericin B	0.01g
Final pH (at 25°C)	7.4±0.2

M1592 - Bolton Broth Base

Ingredients	g / L
Peptone#	10.000
Lactalbumin hydrolysate	5.000
Yeast extract	5.000
Sodium chloride	5.000
Sodium metabisulphite	0.500
Sodium carbonate	0.600
Hemin	0.010
α-ketoglutaric acid	1.000
Sodium pyruvate	0.500

FD231-Bolton Selective Supplement-2 vials

Cefoperazone	10mg
Vancomycin	10mg
Trimethoprim	10mg
Amphotericin B	5mg
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Enzymatic digest of animal tissues

Directions

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.

Principle And Interpretation

Campylobacter species cause mild to severe diarrhoea, with loose, watery stools often followed by bloody diarrhoea. *C. jejuni, C.coli* and *C.lari* are responsible for the major infections. Foods of animal origin are the primary vehicles of *Campylobacter* infections in humans. Unpasteurized milk has been by far the most commonly implicated vehicle in the foodborne outbreaks of *Campylobacter jejuni* enteritis (1,2). *Campylobacter's* were originally classified within the genus *Vibrio*, but they differ from *Vibrio*'s in their DNA Base composition and their ability to grow under conditions of reduced oxygen tension. Selective media were originally designed to isolate *C. jejuni* from faeces, by use of a cocktail of antibiotics in a rich basal medium (3). Bolton Broth Base is formulated as per recommendations of ISO and FDA BAM for detection and enumeration of *Campylobacter* spp. from food and environmental samples in food production area and also from water samples (4-7). This medium is formulated by Bolton (8).

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 gram-positive and gram-negative bacteria while amphotericin B largely reduces the growth of yeasts and moulds.

Type of specimen

Food and animal feeding stuffs; water samples

Specimen Collection and Handling:

Processesing : (4,5,6,7)

Test portion and initial suspension:

Selective Enrichment A : To prepare the initial suspension, take of 10g or 10ml of the test portion with 90ml of the enrichment medium Bolton Broth. Incubate the initial suspension in a micro-aerobic atmosphere at 37°C for 5± 1 hours and then at 41.5°C for 44 hours ± 4 hours.

Plating out : Using the culture obtained in the enrichment medium, inoculate with a sterile 10 µl loop on the surface of mCCD Agar (M887I). Incubate the plates at 41.5°C in a micro-aerobic atmosphere for 44 hours ± 4 hours.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate
3. Biochemical and serological tests are performed for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal medium : Brownish yellow coloured clear to slightly opalescent solution. After addition of lysed horse blood: Red to brown coloured opaque solution in tubes.

Reaction

Reaction of 2.76% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : 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 M887I 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 M887I at 41.5°C in a micro-aerobic atmosphere for 44 hours ± 4 hours.

Organism	Inoculum (CFU)	Recovery on M887I - mCCD Agar	Growth on M887I - mCCD Agar
Productivity			
Campylobacter jejuni ATCC 33291 (00005*) + *Escherichia coli* ATCC 25922 (00013*) + *Proteus mirabilis* ATCC 29906 (00023*)	50-100 >=10^4 >=10^4	>10 colonies	greyish, flat and moist colonies, may have metallic sheen inhibited
Campylobacter jejuni ATCC 29428 (00156*) + *Escherichia coli* ATCC 8739 (00012*) + *Proteus mirabilis* ATCC 29906 (00023*)	50-100 >=10^4 >=10^4	>10 colonies	greyish, flat and moist colonies, may have metallic sheen
Campylobacter coli ATCC 43478 (00004*) + *Escherichia coli* ATCC 25922 (00013*) + *Proteus mirabilis* ATCC 29906 (00023*)	50-100 >=10^4 >=10^4	>10 colonies	greyish, flat and moist colonies, may have metallic sheen

Campylobacter coli ATCC 43478 (00004*) + *Escherichia coli* ATCC 8739 (00012*) + *Proteus mirabilis* ATCC 29906 (00023*)	50-100 >=10^4 >=10^4	>10 colonies	greyish, flat and moist colonies, may have metallic sheen
Selectivity			
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	
Proteus mirabilis ATCC 29906 (00023*)	>=10^4	inhibited	

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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