CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON
BOLTON CCDA ANTIMICROBIC SUPPLEMENT
CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON)

Base medium, selective supplement and ready to use plates for the isolation of *Campoylobacter* spp. in foodstuffs and other samples

TYPICAL FORMULAS

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (CCDA BOLTON)

Beef Extract	10.00
Peptone	10.00
Tryptone	3.00
Sodium Chloride	5.00
Charcoal	4.00
Sodium Desoxycholate	1.00
Ferrous Sulphate	0.25
Sodium Pyruvate	0.25
Agar	15.00

Bolton CCDA Antimicrobic Supplement (vial contents)

Cefoperazone	16 mg
Amphotericin B	5 mg

CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON) ready to use plates

Campy. Blood Free Med. Base Bolton	1000 ml
Cefoperazone	32 mg
Amphotericin B	10 mg

pH: 7.4 ± 0.1

Directions

Suspend 24.2 g of Campylobacter Blood Free Medium Base CCDA Bolton in 500 ml of distilled water; heat to boiling with agitation and autoclave at 121°C for 15 minutes. Cool to 50°C and add the contents of one vial of Bolton CCDA Antimicrobic Supplement (code 4240020). Distribute into sterile Petri dishes with frequent stirring.
Final pH: 7.4 ± 0.1

Description

Campylobacter Blood Free Medium Base CCDA Bolton is prepared according to Bolton, Hutchinson and Coates's formulation and it is used with the Cefoperazone/Amphoterin B Supplement for the isolation of thermophylic *Campylobacter* from clinical specimens on a blood free plating medium. The CCDA (Casein Hydrolysate, Cefoperazone, Desoxycholate Agar) Medium was compared with other six selective substrates for isolation of *C.jejuni* from the faeces (Butzler, Blaser Wang, Skirrow, Preston, Modified Preston, Butzler Virion) by Merino et al. The results indicate a better growth of *C. jejuni* on a CCDA Medium and a less development of contaminating flora. Bolton et al. (1986) also report better percentages in the isolation of *C. jejuni* and *C. coli* from faeces with the CCDA medium.

Technique

For the isolation and identification of thermophylic *Campylobacter* from clinical or foods specimens the following techniques are recommended:

A: Samples with a high Campylobacter contents

A-1: Emulsify the sample in 5 ml of Peptone Water
A-2: Inoculate onto the selective media as described in C-1 and C-2

B: Enrichment for samples with low Campylobacter contents

B-1: Prepare a sample 1:10 dilution in Campylobacter Enrichment Broth
B-2: Incubate at 42°C for 18 hours in a microaerophylic atmosphere (5-6%% oxygen, 10% carbon dioxide and 84-85% nitrogen).

C: Plating

C-1: Inoculate the emulsified or enriched sample onto the selective plating media.
Use at least two selective media: choose the first between the most selective media (Karmali, Bolton, Preston formulations), the second between the less selective media (Skirrow or Blaser Wang). The

bacteriologist must have a good experience as well as knowledge of the bacterial contents of the samples (yeast and moulds and/or Gram-negative bacteria and/or Gram-positive bacteria and/or *Pseudomonas)* to make the best choice.
C-2: Incubate at 42°C in a microaerophilic atmosphere for 48 hours.

D: Presumptive identification

D-1: Pick 2-5 suspected colonies with typical morphology (small or mucoid, usually, greyish, flat with irregular edges spreading or swarming along the inoculation streak) and suspend into 1 ml of Brucella Broth.
D-2: Examine with a phase contrast microscopy to show small slender, curved to spiral rods with a typical darting motility.
D-3; Inoculate from the suspension onto a Columbia Blood Agar plate to get well-isolated colonies and incubate at 42°C in microaerophilic conditions.
D-4: Evaluate the cell morphology with a Gram staining.
D-5: Pick some well-isolated colonies on Columbia Blood Agar and inoculate a tube of Brucella Broth. Incubate at 25°C for 2-5 days.
D-6: Make the catalase and oxidase tests. *Campylobacter* is catalase and oxidase positive.

E: Complete identification

E-1: Evaluate the carbohydrate fermentation in TSI
E-2: Detect the susceptibility/resistance to cephalotin 30 mcg/disc and nalidixic acid 30 mcg/disc on Mueller Hinton Agar supplemented with 5% defibrinated sheep blood.
E-3: Carry out the hippurate test following the usual methods.

The criteria for the identification of thermophylic *Campylobacter* and for the differentiation between species are indicated in the following tables.

TABLE 1 - Main characteristics of thermophylic *Campylobacter*

Cell morphology	small, slender, curved to spiral, Gram-negative rods
Motility	typical darting motility
Growth at 25°C	negative
Oxidase	positive
Catalase	positive
Acid/gas in TSI	negative
Cephalotin	resistant

TABLE 2 - Differential tests for *C. jejuni, C. coli, C. lari*

Test	***C. jejuni***	***C. coli***	***C.lari***
H_2S in T.S.I.	-	-	-
Nalidixic Acid	*S*	*S*	*R*
Hippurate Hydrolysis	+	-	-

S = susceptible R = resistant

User quality assurance (48 h-42°C, reduced O_2)
Productivity control
C.jeuni ATCC 33291*: growth
Selectivity control
E.coli ATCC 25922*: partially or completely inhibited
*NCCLS M22-A2 recommended strains

Storage
Dehydrated medium: 10-30°C
Selective Supplement: 2-8°C
Ready to use plates: 2-8°C
User prepared plates: up to 7 days at 2-8°C

References

• Penner, J.L. (1988) Clin. Microbiol. Rev., **1**, 157 - 172.
• Bolton, F.J., Hutchinson, D.N., Coates, D. (1984) Eur. J. Clin. Microbiol., **5**, 466-468
• Bolton, F.J., Hutchinson, D.N., Coates, D. (1986) J. App. Bacteriol. **56,** 151-157

• ISO 10272:2002 Microbiology of food and animal feeding stuffs - Horizontal method for detection of thermotolerant *Campylobacter*.
• Merino, F.J. et al. (1986) J. Clin. Microbiol. **24**, 451-452

Packaging

Powdered medium

4012822 Campylobacter Blood Free Medium Base Bolton, **500 g (10,3 l)**

Selective Supplement

4240020 Bolton CCDA Antimicrobic Supplement,, **10 vials, each for 500 ml of medium**

Ready to use plates

541113 Campylobacter Blood Free Agar (CCDA Bolton), 20 plates