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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION		
BOLTON BROTH CM0983		

BOLTON BROTH

CM0983

Typical Formula*

Meat peptone	grams per litre	10.0
Lactalbumin hydrolysate		5.0
Yeast extract		5.0
Sodium chloride		5.0
Sodium pyruvate		0.5
Alpha-Ketoglutaric acid		1.0
Sodium metabisulphite		0.5
Sodium carbonate		0.6
Haemin		0.01

* adjusted as required to meet performance standards

Directions

Dissolve 13.8g in 500ml of distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25ml Laked Horse Blood (SR0048) and 1 vial of Bolton Broth Selective Supplement (SR0183E) reconstituted as directed. Mix well and aseptically distribute into sterile containers.

Physical Characteristics

Straw, free-flowing powder
 Colour on reconstitution - straw 3
 Moisture level - less than or equal to 7%
 pH 7.4 ± 0.2 at 25°C
 Clarity - clear

Microbiological Tests Using Optimum Inoculum Dilution

Control Media: Campylobacter Blood-Free Selective Medium or Tryptone Soya Agar, where appropriate

Tested with the addition of Bolton Broth Selective Supplement (SR0183) and enriched with 5% v/v Laked Horse Blood (SR0048)

Inoculate 20ml quantities of medium to achieve 1E+04-1E+06 colony-forming units/20ml (cfu/20ml). Incubate broths aerobically at 42 ± 2°C for 48 ± 4 hours. After incubation, subculture

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broths onto *Campylobacter* Blood-Free Selective Medium (CM0739 + SR0155) and incubate plates under microaerophilic conditions at $42 \pm 2^\circ\text{C}$ for 48 ± 4 hours.

Reactions after microaerophilic incubation at $42 \pm 2^\circ\text{C}$ for 48 ± 4 hours

<i>Campylobacter coli</i>	ATCC®33559	0.5-3 mm grey colonies
<i>Campylobacter lari</i>	ATCC®35221	0.5-3 mm grey colonies
<i>Enterococcus faecalis</i>	ATCC®29212	No growth
<i>Pseudomonas aeruginosa</i>	ATCC®25668	No growth
<i>Pseudomonas aeruginosa</i>	ATCC®27853	No growth
<i>Proteus mirabilis</i>	NCTC10975	No growth
<i>Staphylococcus aureus</i>	ATCC®25923	No growth
<i>Candida albicans</i>	ATCC®10231	No growth or ppt-0.5 mm feather-edged, grey colonies
<i>Aspergillus flavus</i>	ATCC®22547	No growth

Testing performed in accordance with ISO11133:2014

FOOD MICROBIOLOGY TABLE E

Reactions after microaerophilic incubation at $37 \pm 2^\circ\text{C}$ for 5 hours followed by $41.5 \pm 2^\circ\text{C}$ for 44 ± 4 hours.

Mixed Cultures

Inoculate 20ml quantities of medium to achieve 10 to 100 colony-forming units/20ml (cfu/20ml) of *Campylobacter* spp. and $1\text{E}+04$ to $1\text{E}+05$ cfu of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at $37 \pm 2^\circ\text{C}$ for 5 hours followed by $41.5 \pm 2^\circ\text{C}$ for 44 ± 4 hours. After incubation, subculture onto *Campylobacter* Blood-Free Selective Medium (CM0739 + SR0155) and incubate plates under microaerophilic conditions at $41.5 \pm 2^\circ\text{C}$ for 48 hours.

<i>Campylobacter jejuni</i>	ATCC®29428	WDCM00156	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter jejuni</i>	ATCC®29428	WDCM00156	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter jejuni</i>	ATCC®33291	WDCM00005	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

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<i>Campylobacter jejuni</i>	ATCC®33291	WDCM00005	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

<i>Campylobacter coli</i>	ATCC®43478	WDCM00004	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

<i>Campylobacter coli</i>	ATCC®43478	WDCM00004	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

A satisfactory result is represented by recovery of >100 cfu of *Campylobacter* spp. on Campylobacter Blood-Free Selective Medium (CM0739 + SR0155).

Pure Cultures

Inoculate 20ml quantities of medium to achieve 1E+04 to 1E+05 colony-forming units/20ml (cfu/20ml) of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at 37 ± 2°C for 5 hours followed by 41.5 ± 2°C for 44 ± 4 hours. After incubation, subculture onto Campylobacter Blood-Free Selective Medium (CM0739 + SR0155) and incubate plates under microaerophilic conditions at 41.5 ± 2°C for 48 hours.

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
<i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

A satisfactory result is represented by no growth of *Escherichia coli* and *Proteus mirabilis* on Campylobacter Blood-Free Selective Medium (CM0739 + SR0155).

Inoculate 20ml quantities of medium to achieve 1E+04 to 1E+05 colony-forming units/20ml (cfu/20ml) of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at 37 ± 2°C for 5 hours followed by 41.5 ± 2°C for 44 ± 4 hours. After incubation, subculture onto Tryptone Soya Agar (CM0131) and incubate plates under microaerophilic conditions at 37 ± 2°C for 48 hours.

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	1-3mm cream colonies
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	1-3mm cream colonies
<i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	1-2mm straw, swarming colonies

A satisfactory result is represented by <100 cfu of *Escherichia coli* and <10 cfu of *Proteus mirabilis* on Tryptone Soya Agar (CM0131).

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WATER MICROBIOLOGY TABLE F

Reactions after microaerophilic incubation at $37 \pm 2^{\circ}\text{C}$ for 44 ± 4 hours

Mixed Cultures

Inoculate 20ml quantities of medium to achieve 10 to 100 colony-forming units/20ml (cfu/20ml) of *Campylobacter* spp. and $1\text{E}+04$ to $1\text{E}+05$ cfu of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at $37 \pm 2^{\circ}\text{C}$ for 44 ± 4 hours. After incubation, subculture onto Campylobacter Blood-Free Selective Medium (CM0739 + SR0155) and incubate plates under microaerophilic conditions at $41.5 \pm 2^{\circ}\text{C}$ for 48 hours.

<i>Campylobacter jejuni</i>	ATCC®29428	WDCM00156	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter jejuni</i>	ATCC®29428	WDCM00156	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter jejuni</i>	ATCC®33291	WDCM00005	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter jejuni</i>	ATCC®33291	WDCM00005	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter coli</i>	ATCC®43478	WDCM00004	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth
<i>Campylobacter coli</i>	ATCC®43478	WDCM00004	0.5-3.0mm grey colonies
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

A satisfactory result is represented by recovery of >100 cfu of *Campylobacter* spp. on Campylobacter Blood-Free Selective Medium (CM0739 + SR0155).

Pure Cultures

Inoculate 20ml quantities of medium to achieve $1\text{E}+04$ to $1\text{E}+05$ colony-forming units/20ml (cfu/20ml) of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at $37 \pm 2^{\circ}\text{C}$ for 44 ± 4 hours.

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After incubation, subculture onto Campylobacter Blood-Free Selective Medium (CM0739 + SR0155) and incubate plates under microaerophilic conditions at $41.5 \pm 2^{\circ}\text{C}$ for 48 hours.

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
<i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	No growth

A satisfactory result is represented by no growth of *Escherichia coli* and *Proteus mirabilis* on Campylobacter Blood-Free Selective Medium (CM0739 + SR0155).

Inoculate 20ml quantities of medium to achieve $1\text{E}+04$ to $1\text{E}+05$ colony-forming units/20ml (cfu/20ml) of *Escherichia coli* and *Proteus mirabilis*. Incubate broths at $37 \pm 2^{\circ}\text{C}$ for 44 ± 4 hours. After incubation, subculture onto Tryptone Soya Agar (CM0131) and incubate plates under microaerophilic conditions at $37 \pm 2^{\circ}\text{C}$ for 48 hours.

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	1-3mm cream colonies
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	1-3mm cream colonies
<i>Proteus mirabilis</i>	ATCC®29906	WDCM00023	1-2mm straw, swarming colonies

A satisfactory result is represented by <100 cfu of *Escherichia coli* and <10 cfu of *Proteus mirabilis* on Tryptone Soya Agar (CM0131).

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Revision History

Section / Step	Description of Change	Reason for Change	Reference
Creation of ISO11133 section	Update to include testing of ISO11133:2014	Change control	BT-CC-1206
Entire Document	Update to new document format and correction of typographical/minor errors. Removal of OXOID manual. Addition of or ppt-0.5mm feather-edged, grey colonies to <i>Candida albicans</i> ATCC® 10231. Added colour on reconstitution. Corrected typo of ATCC® 33291 to ATCC® 43478 for <i>C. coli</i> .	Change control	BT-CC-2263