



# PRODUCT SPECIFICATION SHEET

## Bolton Broth Base (DM1043)

### Intended Use

Bolton Broth Base (DM1043) is recommended for selective enrichment of *Campylobacter* species from food and animal feed.

### Product Summary and Explanation

*Campylobacters* were originally classified within the genus *Vibrio*, but they differ from *Vibrios* in their DNA Base composition and their ability to grow under conditions of reduced oxygen tension. *Campylobacter* spp. are microaerophilic, very small, curved, thin, Gram-negative rods.<sup>(1)</sup> Microaerophilic organisms have a tendency to be more sensitive to toxic forms of oxygen.<sup>(2)</sup> *Campylobacter* spp. can cause mild to severe diarrhea, with loose, watery stools often followed by bloody diarrhea. These pathogens are highly infective, and transmitted by contaminated food or water. Animal origin foods are the primary vehicles of *Campylobacter* infections in humans. The most commonly implicated vehicle in the foodborne outbreaks of *Campylobacter jejuni* enteritis is unpasteurized milk.<sup>(3,4)</sup> To isolate *Campylobacter jejuni* from faeces, selective media were originally designed by using cocktail of antibiotics in a rich basal medium.<sup>(5)</sup> Bolton Broth Base is formulated as per recommendations of ISO for the selective enrichment of *Campylobacter* species from foods.<sup>(6-8)</sup> Addition of the antibiotics cefoperazone, vancomycin, trimethoprim and amphotericin B (added as freeze dried) makes the medium selective for *Campylobacters*.

### Principles of the Procedure

Bolton Broth Base contains enzymatic digest of animal tissues, lactalbumin hydrolysate and yeast extract which provides nitrogen, carbon, amino acids, and vitamins required which aid resuscitation of sublethally damaged cells of *Campylobacter*. Hemin provides essential growth factors. Sodium Chloride maintains the osmotic balance of the medium. Sodium pyruvate, sodium metabisulphite, and sodium carbonate increase the aerotolerance of *Campylobacter* spp. by acting as oxygen scavengers. Hence microaerophilic incubation is not needed. 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.

### Formula / Liter

Ingredients	Gms / Liter
Enzymatic digest of animal tissues	10.00
Lactalbumin hydrolysate	5.00
Yeast extract	5.00
Sodium chloride	5.00
Sodium metabisulphite	0.50
Sodium carbonate	0.60
Hemin	0.01
alpha-ketoglutaric Acid	1.00
Sodium pyruvate	0.50
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.





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## Directions

1. Suspend 13.8 grams of medium in 500 ml of distilled water.
2. Heat, if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Bolton Selective supplement (MS108) and 25 ml of sterile lysed defibrinated horse blood in the medium.
5. Horse blood may be saponin lysed or lysed by freezing then thawing out.
6. Mix well and aseptically dispense into sterile tubes.

## Quality Control Specifications

<b>Dehydrated Appearance</b>	Light yellow to brownish yellow homogeneous free flowing powder
<b>Prepared Medium</b>	Basal medium : Brownish yellow coloured clear to slightly opalescent solution. After addition of lysed horse blood: Red to brown coloured opaque solution in tubes.
<b>Reaction of 2.76% solution</b>	pH 7.4 ± 0.2 at 25°C
<b>Gel Strength</b>	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed with added Bolton Selective Supplement (MS108) after an incubation at 35-37°C for 4-6 hours and then at 41.5°C for 40-48 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Candida albicans</i> ATCC 10231	>=10 <sup>3</sup>	inhibited
2.	<i>Campylobacter coli</i> ATCC 33559	50-100	good-luxuriant
3.	<i>Campylobacter jejuni</i> ATCC 29428	50-100	good-luxuriant
4.	<i>Escherichia coli</i> ATCC 25922	>=10 <sup>3</sup>	inhibited

The organisms listed are the minimum that should be used for quality control testing.

## Test Procedure

1. Inoculate a small quantity of the test sample into nine times its volume of Bolton Broth (DM1043), so as to obtain a test portion / enrichment medium ratio of 1:10 (mass/volume or volume/volume), and homogenize.
2. Incubate at 37°C for 4-6 hours, then at 41.5°C for 44 ± 4 hours.
3. A loopful of this enriched culture is streaked on Karmali Campylobacter Agar Base (DM1388).
4. Refer to appropriate references for standard test procedures.

## Results

Refer to appropriate references and standard test procedures for interpretation of results.

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.





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### Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

Product Name : Bolton Broth Base

Product Code : DM1043

Available Pack sizes : 500gm

### References

1. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8<sup>th</sup> ed., AOAC International, Gaithersburg, MD.
2. George, H. A., P. S. Hoffman, and N. R. Krieg. 1978. J. Clin. Micro. 8:36-41.
3. Blasser M.J., Cravens J., Powers B.W., LaForce F.M., and Wang W. L.L., 1979, Am. J. Med., 67:715.
4. Brieseman M.A., 1984, N.Z. Med. J., 97:411.
5. Corry, Curtis and Baird. Culture Media For Food Microbiology, Vol.34. Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam.
6. Hunt J.M, Campylobacter, F.D.A Bacteriological Analytical Manual, 8th Edition (Revision AOAC, Arlington V A (1998).
7. Bolton F. J., Personal communication (1995).
8. International Organization for Standardization (ISO), 2006, Draft ISO 10272- 1:2006 (E).

### Further Information

For further information please contact your local MICROMASTER Representative.



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