

## Campy-Blood Free Selective Medium (CCDA) (NCM0042)

### Intended Use

Campy Blood-Free Selective Medium (CCDA) is used with cefoperazone for the selective isolation of *Campylobacter* spp. in a laboratory setting. Campy Blood-Free Selective Medium (CCDA) is not intended for use in the diagnosis of disease or other conditions in humans

### Description

Campy Blood-Free Selective Medium (CCDA) was described by Bolton et al. This medium was formulated to replace blood with charcoal, ferrous sulfate, and sodium pyruvate. To improve selectivity, cefoperazone replaced cephalosporin in the original formulation. Bolton et al recommended incubating inoculated plates at 37°C to improve isolation rates. Yeast and fungal contaminants are inhibited with the addition of amphotericin B.

Campy Blood-Free Selective Medium (CCDA) is recommended for food testing.

### Typical Formulation

Nutrient Broth No. 2	25.0 g/L
Charcoal	4.0 g/L
Casein Acid Hydrolysate	3.0 g/L
Sodium Deoxycholate	1.0 g/L
Ferrous Sulfate	0.25 g/L
Sodium Pyruvate	0.25 g/L
Agar	12.0 g/L

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Supplement

NCM4069     Campylobacter Blood Free (CFP)

### Precaution

Refer to SDS

### Preparation

1. Suspend 45.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45 - 50°C and aseptically add 2 vials of reconstituted NCM4069-0.5\* supplement, each reconstituted using 5mL of sterile deionized/RO water,
5. Mix well and pour into petri dishes.

\*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and grey-black.

**Prepared Appearance:** Prepared medium is grey-black and opaque.

**Expected Cultural Response:** Cultural response on Campy Blood-Free Selective Medium (CCDA) at 37°C after 18 - 48 hours in an atmosphere consisting of approximately 5 - 6% oxygen, 3 - 10% carbon dioxide, and 84 - 85% nitrogen.

Microorganism	Inoculum (CFU)	Response
<i>Campylobacter jejuni</i> ATCC® 33291	10-300	Good Growth
<i>Escherichia coli</i> ATCC® 25922	~1000	Completely Inhibited

The organisms listed are the minimum that should be used for quality control testing. Note: Quality Control Laboratory sample was tested with the addition of cefoperazone.

## **Test Procedure**

1. Inoculate the specimen directly onto the surface of the prepared Campy Blood-Free Selective Medium (CCDA). If an enrichment broth is required, refer to the appropriate references.
2. Streak for isolation.
3. Incubate inoculated plates at 37°C or 42°C in an atmosphere composed of 5 - 6% oxygen, 3 - 10% carbon dioxide and 84 - 85% nitrogen for 24 - 48 hours. Selective temperatures are required for certain *Campylobacter* spp. Refer to appropriate references on the proper temperature for the targeted *Campylobacter* spp.

## **Results**

*Campylobacter* colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora is completely to markedly inhibited.

Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.

## **Expiration**

Refer to 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.

## **Limitation of the Procedure**

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

## **Storage**

Store dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

## **References**

1. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm)
2. Bolton, F. J., D. N. Hutchinson, and D. Coates. 1984. J. Clin. Microbiol. 19:169-171.
3. Bolton, F. J., and D. N. Hutchinson. 1984. J. Clin. Pathol. 34:956-957.
4. Bolton, F. J., D. N. Hutchinson, and G. Parker. 1988. Eur. J. Clin. Microbiol. Infect Dis. 7:155-160.
5. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of food, 4<sup>th</sup> ed. American Public Health Association, Washington, D.C.
6. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.