CHARCOAL SELECTIVE MEDIUM

INTENDED USE

Remel Charcoal Selective Medium is a solid medium recommended for use in qualitative procedures for primary isolation of *Campylobacter* species from clinical specimens.

SUMMARY AND EXPLANATION

In 1984, Bolton et al. reported charcoal could effectively replace blood in a culture medium for isolating *Campylobacter*.[1] In further testing, they demonstrated improved recovery of *Campylobacter* using a medium with cefoperazone in place of cefazolin.[2] In 1986, Karmali used a medium containing cefoperazone, vancomycin, and cycloheximide to demonstrate improved selectivity and a higher recovery rate of *Campylobacter* spp. when compared to Skirrow's medium.[3] Gun-Munro et al. evaluated six selective media using 2,780 human, animal, and avian fecal specimens.[4] They reported modified charcoal cefoperazone desoxycholate agar (CCDA) demonstrated enhanced isolation of *Campylobacter* spp. and greater suppression of commensal microbial flora as compared to the other media tested. In 1988, Griffiths further evaluated CCDA and found it to be superior to other media tested in recovering *Campylobacter* and reducing the growth of contaminating organisms.[5] Endtz et al. confirmed these findings in 1991, reporting a higher isolation rate for *Campylobacter* when using Charcoal Selective Medium.[6]

PRINCIPLE

Beef extract supplies nitrogen, carbohydrates, vitamins and other nutrients required for the growth of *Campylobacter.* Gelatin and casein peptones provide nutrients in the form of amino acids and peptides. Charcoal is a detoxifying agent and reduces oxygen tension. Sodium chloride supplies essential electrolytes and maintains osmotic equilibrium. Ferrous sulfate and sodium pyruvate enhance the growth and aerotolerance of *Campylobacter* spp. Sodium desoxycholate is a selective agent which inhibits some bacteria. Cefoperazone is a broad-spectrum cephalosporin with enhanced activity against pseudomonads and members of the family *Enterobacteriaceae*. Vancomycin is added to inhibit gram-positive organisms and cycloheximide to inhibit fungi and yeast. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

Beef Extract	10.0	g	Sodium Pyruvate	0.25	g
Gelatin Peptone	10.0	g	Cycloheximide	100.0	mg
Sodium Chloride	5.0	g	Cefoperazone	32.0	mg
Charcoal	4.0	g	Hematin	32.0	mg
Casein Peptone	3.0	g	Vancomycin	20.0	mg
Sodium Desoxycholate	1.0	g	Agar	12.0	g
Ferrous Sulfate	0.25	g	Demineralized Water	1000.0	ml

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

Note: Specimens for the isolation of *Campylobacter* spp. should be placed in transport medium when a delay in processing of more than 2 hours is anticipated or when a rectal swab is collected. Optimum recovery of *Campylobacter* spp. from stool specimens is achieved by using a combination of selective media.[7]

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory. Rectal swabs and liquid stools can be inoculated directly onto Charcoal Selective Medium. Formed stool specimens should be emulsified in sterile saline (0.85%) prior to inoculation. Place 1 or 2 drops of liquid or formed stool suspension onto agar and streak for isolation.
2. Incubate plate(s) in a microaerophilic environment (mixture of 5% O_2, 10% CO_2, 85% N_2) for 48-72 hours at 40-42°C. Media may be set in duplicate and incubated at 33-37°C as well as 40-42°C to allow for the growth of certain *Campylobacter* spp.
3. Observe for characteristic colonies which can be flat, irregular, or spreading on fresh medium. Some strains appear as a thin film on the agar or form colonies that tail along the line of streaking. On less fresh media, colonies are 1 to 2 mm in diameter, round, convex, and glistening. Colonies can be yellowish to gray or pinkish in color and are nonhemolytic.

QUALITY CONTROL

All lot numbers of Charcoal Selective Medium have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[8] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Campylobacter coli ATCC® 33559	Microaerophilic, up to 48 h @ 40-42°C	Good growth
**Campylobacter jejuni* ATCC® 33291	Microaerophilic, up to 48 h @ 40-42°C	Good growth
Cryptococcus neoformans ATCC® 34877	Ambient, up to 72 h @ 25-30°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Proteus mirabilis ATCC® 12453	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Pseudomonas aeruginosa ATCC® 27853	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

*CLSI recommended organism

LIMITATIONS

1. Extending incubation to 72 hours may increase the isolation rate of *Campylobacter* spp.[3]
2. *Campylobacter coli*, *Campylobacter fetus*, and some strains of *Campylobacter jejuni* are inhibited by cephalosporins.[9]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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