

Brazier's CCEY Agar

BC2160

A development from Anaerobe Agar (BC2090), Braziers CCEY Agar incorporates additional ingredients to improve the isolation and differentiation of *C.difficile* from clinical specimens. The medium is used by the Anaerobe Reference Unit in Cardiff for the isolation of *C.difficile*, resulting from work initiated by Ken Phillips and Paul Levett, and completed by Jon Brazier. Cholic acid is present to promote spore germination following alcohol shock treatment, and p-hydroxyphenylacetic acid to enhance the production of p-cresol, a distinctive metabolite of *C.difficile*. The medium is made selective by the addition of S2093 (cefoxitin/cycloserine) and egg yolk emulsion is added to help differentiate *C.difficile* from lecithinase positive clostridia. With the addition of lysed horse blood fluorescence under UV light can be used as an aid to identification.

Formula grams per litre

Peptone Mix	23.0
Sodium chloride	5.0
Soluble Starch	1.0
Cysteine HCl	0.5
Haemin	0.01
Vitamin K	0.001
L-arginine	1.0
Soluble pyrophosphate	0.25
Sodium succinate	0.5
Cholic acid	1.0
p-Hydroxyphenylacetic acid	1.0
Bacteriological Agar	12.0
Sodium bicarbonate	0.4
Glucose	1.0
Sodium pyruvate	1.0

minutes, swirl to mix, and sterilise by autoclaving at 121⁰C for 15 minutes. Cool to 47⁰C and add 2 vials of S2093, 40ml of Egg Yolk Emulsion S2073 and 10ml lysed horse blood. Mix well and pour into petri dishes.

pH: 7.0 +/- 0.2

Appearance: Tan opaque gel.

Preparation

Suspend 48 grams of powder in 1 litre of deionised water. Allow to soak for 10

Storage of Prepared Medium

Plates should be stored at 2-8⁰C in the dark. Plates should be used within 1 week.

Quality Control Organisms - Suggestions

<i>C.difficile</i>		
<i>E.coli (inhibition)i</i>	ATCC 11775	

Directions for use

Surface streak untreated or alcohol shocked specimens for single colonies. Incubate at 37⁰C under anaerobic conditions for 24-48hrs.

Continued

Characteristics of C.difficile:

Gray opaque flat colonies, raised elevation, 2-3mm diameter, generally circular but tending to elongate in the direction of spreading, ground glass appearance and a rough, fimbriate edge. Lecithinase negative. Incubation longer than 48 hrs may result in a lighter gray or white centre to the colony. Phenolic odour due to the production of p-cresol. Colonies fluoresce yellow-green under UV light. Confirm by latex agglutination.

References:

Brazier J S (1993) Role of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) S228-33.