

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

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|----------------------------|-------|
| 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

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|-----------------------------|------------|--|
| <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.