PRODUCT SPECIFICATION

CE

Product Name	**Brazier's Clostridium difficile Selective Medium, modified**
Product Code	**PB5191A**

Form of Product	Poured plate
Storage	6 – 12°C, dark
Filling weight	17 g ± 5 %
Packaging	10 plates wrapped in foil
pH	7.0 ± 0.2
Colour	Copper brown, opaque
Shelf life	6 weeks
Intended Usage	A medium for the isolation of *Clostridium difficile*. For professional use only.
Technique	Depends on the different methods. For information see Product Information.

Typical Formulation	grams per litre
Peptone mix	23.0
Sodium chloride	5.0
Soluble starch	1.0
Sodium bicarbonate	0.4
Glucose	1.0
Sodium pyruvate	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
Cefoxitin	0.008
D-Cycloserine	0.25
Amphotericin	0.008
Neutral red	0.03
Egg yolk	40.0 ml
Defibrinated Horse blood	10.0 ml
Agar	12.0

Quality Control

1. Control for general characteristics, labelling and printing.

2. Control for sterility
   ≥ 72 h @ 25 ± 1°C, aerobic
   ≥ 72 h @ 36 ± 1°C, aerobic

3. Biological control
   Inoculum size for productivity: 10 - 100 cfu per plate
   Inoculum size for selectivity: 10^4 – 10^5 cfu per plate

   Incubation conditions: 40 – 48 h @ 36 ± 1°C, anaerobic

Control Strain	Growth
Clostridium difficile ATCC 9689	2 – 5 mm, dark grey colonies; lecithinase negative; Green-yellow fluorescence under UV light.
Bacteroides fragilis ATCC 25285	Complete inhibition (≤ 10 cfu).
Candida albicans ATCC 10231	Complete inhibition (≤ 10 cfu).

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APPENDIX

PRODUCT INFORMATION

Product Name	**Brazier's Clostridium difficile Selective Medium, modified**
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Description

Brazier´s CCEY Clostridium difficile Selective Medium, modified, is based on the formulation described by Jon Brazier[1]. It contains ingredients to improve the isolation and differentiation of *C. difficile* from clinical specimens. 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*. Selectivity is achieved by addition of cefoxitin, cyloserine, amphotericin and neutral red. Egg yolk emulsion is added to help to differentiate *C. difficile* from lecithinase positive clostridia. Finally the addition of lysed horse blood optimises the recognition of colony fluorescence when cultures are examined using UV light.

Technique

Inoculate the agar with the material in fractions and incubate for 24-48 hours at 36 ± 1°C under anaerobic conditions.

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 reaction is negative. Incubation longer than 48hrs may result in a lighter gray or white centre to the colony. Phenolic odour due to the production of p-cresol is present. Colonies fluoresce yellow-green under UV light. Confirmation by latex agglutination is needed.

Reference

)[1] Brazier J.S.: Role of the laboratory in investigations of Clostridium difficile diarrhoea. Clinical Infectious Diseases, 1993 June; 16 Supplement 4, pages 228-33.

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