Clostridium Difficile Agar Base (Brazier's)
SKU: 700004515, 700004516, 700004517, 700004518
NCM0128

Intended Use
Clostridium Difficile Agar Base (Brazier's) is used with blood, Cycloserine, and Cefoxitin for the isolation of *Clostridium difficile*. *Clostridium Difficile* is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Brazier's CCEY agar is the formulation currently used by the Anaerobe Reference Unit for the isolation of *C.difficile,* resulting from work initiated by Ken Phillips and Paul Levett, and completed by Jon Brazier. Based upon the market leading anaerobe medium, Fastidious Anaerobe Agar, it incorporates additional ingredients to improve the isolation and differentiation of *C.difficile* from samples.

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 supplement NCM4044 (Cycloserine & Cefoxitin) and Egg Yolk Emulsion 50% NCM4017 is added to help differentiate *C.difficile* from lecithinase positive clostridia. Finally, the addition of lysed horse blood optimizes the recognition of colony fluorescence when cultures are examined using UV light.

Typical Formulation

Peptone Mix	23.0 g/L
Sodium Chloride	5.0 g/L
Soluble Starch	1.0 g/L
Agar	12.0 g/L
Sodium Bicarbonate	0.4 g/L
Glucose	1.0 g/L
Sodium Pyruvate	1.0 g/L
Cysteine HCl	0.5 g/L
Hemin	0.01 g/L
Vitamin K	0.001 g/L
L-Arginine	1.0 g/L
Soluble Pyrophosphate	0.25 g/L
Sodium Succinate	0.5 g/L
Cholic Acid	1.0 g/L
p-Hydroxyphenylacetic acid	1.0 g/L

pH: 7.0 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Supplements
NCM4017 Egg Yolk Emulsion 50%
NCM4044 or 100001648 Cycloserine & Cefoxitin Supplement

Precaution
Refer to SDS

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Preparation

1. Suspend 48 grams 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 to 45-50°C and aseptically add the following: 2 vials of NCM4044-0.5* Cycloserine & Cefoxitin Supplement, each reconstituted using 5 mL of sterile deionized/RO water, 40 mL of NCM4017* Egg Yolk Emulsion 50% and 10-mL lysed horse blood.
5. Mix well and dispense.

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

Test Procedure

For a complete discussion on the isolation and identification of *C. difficile* and other anaerobic bacteria refer to specific procedures in appropriate references.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium with supplements is opaque and brown/red.

Expected Cultural Response: Cultural response on Clostridium Difficile Agar Base supplemented with lysed horse blood, NCM4017 and NCM4044, and inoculated with the organisms listed below. Cultures were incubated at 37 ± 1ºC under the appropriate atmosphere and examined for growth at 48 hours.

Microorganism	Approx. Inoculum (CFU)	Recovery
Bacteroides fragilis ATCC® 25285	$>10^3$	Complete Inhibition
Clostridium difficile ATCC® 17858	50-200	>50%
Clostridium perfringens ATCC® 13124	$>10^3$	Complete Inhibition
Clostridium sporogenes ATCC® 11437	$>10^3$	Complete Inhibition
Escherichia coli ATCC® 25922	$>10^3$	Complete Inhibition
Staphylococcus aureus ATCC® 25923	$>10^3$	Complete Inhibition

The organisms listed are the minimum that should be used for quality control testing.

Results

Colonies of *C. difficile* are 4 - 6 mm in diameter, irregular, raised, opaque, and grey-white after 48 hours incubation.

Expiration

Refer to expiration date stamped on the container. Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Further tests are necessary for confirmation of *C. difficile*.
2. Clostridium Difficile Agar does not contain Neutral Red indicator because it is designed for use with horse blood.

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3. Typical Gram stain morphology of *C. difficile* may not be evident in colonies picked from this medium because of antibiotics present. Subculture suspected colonies to blood agar to obtain characteristic morphology.

Storage

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

References

1. Brazier J.S. (1993) Rôle of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) 228-33.
2. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. George, W. L., V. L. Sutter, D. Citron, and S. M. Finegold. 1979. Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. 9:214.
4. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

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