

CCEY *Clostridioides difficile* Medium

DM373

Intended Use

A basal medium for the selective isolation of *Clostridioides difficile*.

Contents

See pack label.

Formulation*

Material:	Concentration in medium:
Peptone mixture	23.0g/litre
Sodium chloride	5.0g/litre
Soluble starch	1.0g/litre
Agar	12.0g/litre
Sodium bicarbonate	0.4g/litre
Glucose	1.0g/litre
Sodium pyruvate	1.0g/litre
Cysteine HCl	0.5g/litre
Haemin	0.01g/litre
Vitamin K	0.001g/litre
L-arginine	1.0g/litre
Soluble pyrophosphate	0.25g/litre
Sodium succinate	0.5g/litre
Cholic acid	1.0g/litre
p-Hydroxyphenylacetic acid	1.0g/litre
Final pH: 7.0 ± 0.2	

Storage and shelf life

All dehydrated culture media containers should be kept tightly closed and stored in a dry place at 10 to 25°C until the expiry date shown on the pack label.

Precautions

For *in vitro* diagnostic use only. Observe approved hazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet (available on request or via MAST® website).

Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST® selective supplements, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood.

Procedure

1. Refer to pack label for quantities and volumes required. Prepare MAST CCEY *Clostridioides difficile* Medium (DM373D) by suspending the powder in distilled or deionised water. For sachet packs, dissolve the entire contents of the sachet in the volume shown on the label.

2. Allow to stand for 15 minutes and mix well.
3. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
4. Allow to cool to 45 to 50°C, and hold at this temperature in a water bath.
5. Add MAST® SELECTAVIAL (SV23 series) as specified and gently mix.
6. Supplement the medium with 1%v/v of sterile lysed horse blood and 40ml/L of MAST® REDIPREP Egg Yolk Emulsion (DM096S) and mix well.
7. Pour culture plates (15 to 20ml per plate) and allow to set.
8. Prepared culture plates may be used immediately or stored in an upright position in plastic bags at 2 to 8°C for up to two weeks before use.
9. Inoculate plates directly with faecal sample or after enrichment, by surface plating, streaking out for single colonies.
10. Incubate plates anaerobically at 37°C for 24 to 48 hours.

Interpretation of results

After incubation record growth of organisms. Colonies of *Cl. difficile* will grow 1 to 3mm in diameter after 48 hours incubation, appearing grey/white in colour, lecithinase negative with a ground glass appearance and a rough, fimbriate edge. After 48 hours, colonies of *Cl. difficile* can be distinguished from other organisms that occasionally grow on the medium by morphology and phenolic odour. Colonies fluoresce yellow/green under UV light.

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate expected performance. Do not use the product if the result with the control organism is incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organisms	Result
<i>Clostridioides difficile</i> ATCC® 9689	Positive
<i>Clostridium perfringens</i> ATCC® 13124	Negative
<i>Escherichia coli</i> ATCC® 25922	Negative
<i>Enterococcus faecalis</i> ATCC® 29212	Negative

References

Bibliography available on request.