

***C. difficile* Medium**

DM254

Intended Use

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

Contents

See pack label.

Formulation*

Material:	Concentration in medium:
Peptone mixture	30.5g/litre
Yeast extract	1.0g/litre
Casein hydrolysate enzymic	8.5g/litre
Fructose	6.0g/litre
Sodium chloride	5.0g/litre
Potassium dihydrogen phosphate	0.1g/litre
Disodium hydrogen phosphate	0.5g/litre
Magnesium sulphate	0.12g/litre
Agar	12.0g/litre
Final pH: 7.4 ± 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® *C. difficile* Medium (DM254D) 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. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
3. Allow to cool to 50 to 55°C, and hold at this temperature in a water bath.
4. Add *C. difficile* MAST® SELECTAVIAL (SV23 series) as specified and gently mix.

5. Supplement the medium with 5 to 7% sterile defibrinated horse blood and mix well.
6. Pour culture plates (15 to 20ml per plate) and allow to set.
7. 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.
8. Inoculate plates directly with faecal sample or after enrichment, by surface plating, streaking out for single colonies.
9. Incubate plates anaerobically at 37°C for 24 to 48 hours.

Interpretation of results

After incubation record growth of organisms. Colonies of *C. difficile* will grow 4 to 6mm in diameter after 48 hours incubation, appearing yellow in colour. The medium immediately surrounding the colony often becoming orange due to pigmentation. After 24 hours, colonies of *C. difficile* can be distinguished from other organisms that occasionally grow on the medium.

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>C. difficile</i> ATCC® 9689	Positive
<i>C. perfringens</i> ATCC® 13124	Negative
<i>Escherichia coli</i> ATCC® 25922	Negative
<i>Enterococcus faecalis</i> ATCC® 29212	Negative

References

Bibliography available on request.