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T.C.B.S. Cholera Medium

DM218

Intended Use

A selective medium for the isolation of *Vibrio* spp.

Contents

See pack label.

Formulation*

Material:	Concentration in medium:
Selected peptone mixture	17.0g/litre
Yeast extract	5.0g/litre
Sucrose	20.0g/litre
Sodium chloride	10.0g/litre
Sodium citrate	10.0g/litre
Ferric citrate	1.0g/litre
Sodium thiosulphate	10.0g/litre
Bile salts	3.0g/litre
Thymol blue	0.04g/litre
Bromothymol blue	0.04g/litre
Agar A	14.0g/litre
Final pH: 8.6 ± 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® T.C.B.S. Cholera Medium (DM218D) 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 approximately 15 minutes, then bring to the boil until completely dissolved. DO NOT AUTOCLAVE.
3. Allow to cool to 50 to 55°C, mix well, pour culture plates (15 to 20ml per plate) and allow to set.

4. Prepared culture plates may be used immediately or stored in plastic bags at 2 to 8°C for up to one week before use.
5. Inoculate plates with a heavy load of faecal material or a subculture from an enrichment medium (e.g. alkaline peptone water), by surface plating, streaking out for single colonies.
6. Incubate plates aerobically for 18 to 24 hours at 35 to 37°C.

Interpretation of results

After incubation record growth of organisms. Typical characteristics to note include: colony size morphology and pigmentation.

Organism	Colonial appearance
<i>V. cholerae</i>	2-3mm diameter, Yellow
<i>V. parahaemolyticus</i>	2-5mm diameter, Blue-green
<i>V. alginolyticus</i>	2-5mm diameter, Yellow
<i>V. metschnikovii</i>	2-4mm diameter, Yellow
<i>V. fluvalis</i>	2-3mm diameter, Yellow
<i>V. vulnificus</i>	2-3mm diameter, Blue-green
<i>V. mimicus</i>	2-3mm diameter, Blue-green
<i>Enterococcus</i> spp.	1mm diameter, Yellow, normally suppressed
<i>Proteus</i> spp.	1mm diameter, Yellow-green
<i>Pseudomonas</i> spp.	1mm diameter, Blue-green

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>Escherichia coli</i> ATCC® 25922	No Growth
<i>Vibrio cholera</i> ATCC® 11348	Growth, Yellow
<i>Vibrio parahaemolyticus</i> ATCC® 17803	Growth, Blue / Green

Limitations

Due to the complexity of the formulation of this medium, growth of organisms can vary between different batches.

References

Bibliography available on request.