Mast Group Ltd.
Mast House, Derby Road,
Bootle, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

T.C.B.S. Cholera Medium

DM401

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

Contents
See pack label.

Formulation*

Material:	Concentration in medium:
Selected peptone mixture	10.0g/litre
Yeast extract	5.0g/litre
Sucrose	20.0g/litre
Sodium chloride	10.0g/litre
Ferric citrate	1.0g/litre
Sodium thiosulphate	10.0g/litre
Sodium cholate	3.0g/litre
Sodium citrate·2H_2O	10.0g/litre
Oxgall	5.0g/litre
Thymol blue	0.04g/litre
Bromothymol blue	0.04g/litre
Agar	14.0g/litre
Final pH: 8.8 ± 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 (DM401D) 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
V. cholerae	2-3mm diameter, Yellow
V. parahaemolyticus	2-5mm diameter, Blue-green
V. alginolyticus	2-5mm diameter, Yellow
V. metschnikovii	2-4mm diameter, Yellow
V. fluvalis	2-3mm diameter, Yellow
V. vulnificus	2-3mm diameter, Blue-green
V. mimicus	2-3mm diameter, Blue-green
Enterococcus spp.	1mm diameter, Yellow, normally suppressed
Proteus spp.	1mm diameter, Yellow-green
Pseudomonas 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
Escherichia coli ATCC® 25922	No Growth
Vibrio alginolyticus ATCC® 17749	Growth, Yellow
Vibrio parahaemolyticus ATCC® 17803	Growth, Blue / Green

References
Bibliography available on request.

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*Formulation may change to meet performance criteria