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

Kohn's No.2 Medium

DM138-2

Intended Use
A composite medium for the differentiation of Enterobacterales (for use in conjunction with Kohn's No.1 medium DM138-1).

Contents
See pack label.

Formulation*

Material:	Concentration in medium:
Peptone mixture	20.0g/litre
Sucrose	10.0g/litre
Salicin	10.0g/litre
Sodium thiosulphate	0.016g/litre
Di-sodium hydrogen orthophosphate	0.09g/litre
Sodium chloride	5.0g/litre
Bromo-thymol blue	0.02g/litre
Agar	3.0g/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® Kohn's No.2 Medium (DM138-2D) 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. Boil to dissolve completely.
3. Mix well and distribute into test tubes.
4. Autoclave at 115°C (10 p.s.i.) for 15 minutes.
5. Allow to set vertically.
6. Inoculate with a straight wire by stabbing one third the depth of the semi-solid medium.
7. For the detection of Indole and H_2S, impregnated paper strips are required.
8. The Indole and H_2S paper strips can be suspended in the neck of the tube.
9. Incubate tubes at 35 to 37°C for 18 to 24 hours.

Interpretation of results
Sugar fermentation is indicated by a colour change in the bromo-thymol blue indicator, from blue/green at pH7.4 to yellow at pH6.0. Fermentation of salicin, sucrose, or both is indicated by a yellow colour. Weak colour changes to green/light green should be classed as negative. Motility is indicated by a diffuse growth spreading away from the line of inoculation, or by turbidity in the whole medium. H_2S production causes a blackening of the lower part of the lead acetate strip and indole production gives a colour change in the indole strip from yellow to red.

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.

Organism	Fermentation Sucrose/ salicin	Motility	H_2S	Indole
Salmonella typhimurium ATCC® 14028	-	+	±	-
Shigella sonnei ATCC® 25931	-	-	-	-
Proteus mirabilis ATCC® 29906	Acid + gas or -	+	±	±

± = variable reaction

References
Bibliography available on request.

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*Formulation may change to meet performance criteria