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Kohn's No.1 Medium

DM138-1

Intended Use

A composite medium for the differentiation of Enterobacteriales (for use in conjunction with Kohn's No.2 medium DM138-2).

Contents

See pack label.

Formulation*

Material:	Concentration in medium:
Peptone mixture	15.0g/litre
Meat extract	2.0g/litre
Yeast extract	2.0g/litre
Dextrose	1.0g/litre
Mannitol	10.0g/litre
Phenol red	0.05g/litre
Agar	16.0g/litre
Final pH: 7.2 ± 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.1 Medium (DM138-1D) 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 115°C (10 p.s.i.) for 15 minutes.
3. Cool to 60°C.
4. Add 25ml of sterile 40% w/v Urea Solution (DM228S) per litre of medium.
5. Mix well and slope with 1 inch butts.

6. Inoculate slopes with a straight wire from a pure culture, or single colonies taken from solid selective media.
7. Stab deep into the butt and smear the surface of the slope.
8. For the detection of Indole and H₂S, impregnated paper strips are required.
9. The Indole and H₂S paper strips can be suspended in the neck of the tube. Incubate the slope at 35 to 37°C for 18 to 24 hours.

Interpretation of results

The production of acid, aerobically on the surface and anaerobically in the butt is detected by a colour change in the phenol red indicator from yellow at pH 6.8 to cerise at pH 8.4. Fermentation of dextrose only is indicated by a yellow butt with or without gas, and a red slope. A yellow slope indicates mannitol fermentation, while urease positive organisms produce an alkaline reaction, imparting a cerise colour to the whole medium. H₂S 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 of		Urease	H ₂ S	Indole
	Dextrose	Mannitol			
Salmonella typhimurium ATCC® 14028	Acid + gas	Acid	-	±	-
Shigella sonnei ATCC® 25931	Acid	Acid	-	-	-
Proteus mirabilis ATCC® 29906	(-)	(-)	+	±	±

(-) = apparent negative reaction, urease activity masks fermentation reaction

± = variable reaction

References

Bibliography available on request.