MAST® Culture Media and Supplements

Technical Information Sheet

Product Code DM 138

Kohn's No. 1 & 2 Media

Composite media for the differentiation of *Enterobacteriaceae.*

1. Description

Preliminary differentiation of isolates in the examination of faecal specimens has involved the laborious inoculation of numerous sugar containing media. An obvious way of economising in effort and materials has been the use of composite media in which two or more biochemical tests are performed in the same tube or plate. This has enabled preliminary screening of all isolates so that those of low grade pathogenicity can be excluded.[1] Probably the earliest known composite medium was the double sugar agar of Russel[2] in and since then numerous modifications have been developed. There is an obvious limit to the information that can be obtained from one medium and considering this, Kohn[3] developed a two tube media system giving a more complete biochemical profile. Gillies[4] suggested various modifications and most of these have been used in the MAST formulation. colour change in the bromo-thymol blue indicator, from blue green at pH 7.4 to yellow at pH 6.0. 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.

2. Directions

No. 1 medium

1. Suspend by swirling 46g in 1 litre or the contents of the sachet in the stated volume of distilled or deionised water.

2. Autoclave at 115ºC (10p.s.i.) for 15 minutes.

3. Cool to 60ºC

4. Add 25ml of sterile 40% w/v Urea Solution (DM228s) per litre of medium.

5. Mix well and slope with 1 inch butts.

*Formulation may be modified to meet performance criteria

No. 2 medium

1. Suspend by swirling 48g in 1 litre or the contents of the sachet in the stated volume of distilled or deionised water.

2. Boil to dissolve completely

3. Mix well and distribute into test tubes.

4. Autoclave at 115ºC (10p.s.i.) for 15 minutes

5. Allow to set vertically.

3. Typical Formula*

No. 1 medium

Formula	grams per litre
Meat extract	2.0
Peptone mixture	15.0
Mannitol	10.0
Agar	16.0
Yeast extract	2.0
Dextrose	1.0
Phenol red	0.05
pH approx 7.2	

Indole

Strips of filter paper approximately 5x50mm are impregnated with the following solution:-

p - dimethylaminobenzaldehyde	5g
o - phosphoric acid	10ml
Methanol	50 ml

Strips are dried for a minimum period at 50ºC.

No. 2 medium

Formula	grams per litre
Peptone mixture	20.0
Salicin	10.0
Sodium thiosulphate	0.016
Bromo-thymol blue	0.02
Agar	3.0
Sucrose	10.0
Sodium chloride	5.0
Disodium hydrogen orthophosphate	0.09
pH approx 7.4	

H_2S

Impregnate similar strips with saturated lead acetate solution and dry at 70ºC.

*Formulation may be modified to meet performance criteria

4. In Use

No. 1 medium

Inoculate with a straight wire from a pure culture, or single colonies taken from solid selective media. Stab deep into the butt and smear the surface of the slope. Incubate for 18-24 hours at 37ºC, after which time 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.

No. 2 medium

Inoculate with a straight wire by stabbing one third the depth of the semi-solid medium. Suspend the two test papers in the neck of the tube. Incubate at 37ºC for 18-24 hours, after which time sugar fermentation is indicated by a colour change in the bromo-thymol blue indicator, from blue green at pH 7.4 to yellow at pH 6.0. 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.

5. Typical Results (Gilles 1956)[4]

	No. 1 Medium		No. 2 Medium				
	Fermentation of		Urease Production	Fermentation Sucrose/Salicin	Motility	Production of H_2S (black)	Indole Formation (pink/red)
	Dextrose	Mannitol					
S.typhi	A	A	-	-	+	+	-
Other salmonella	AG	A	-	-	+	±	-
Sh.sonnei	A	A	-	-	-	-	-
Sh.flexneri	A	A	-	-	-	-	±
Sh.dysenteriae Serotype 2*	A		-	-	-	-	+
Proteus	(-)	(-)	+	AG or -	+	±	±

AG = acid & gas (-) = apparent negative reaction, urease activity masks effects fermentation
A= acid only ± = variable reaction + = positive - = negative * = Shchmitz Bacillus

*Formulation may be modified to meet performance criteria

6. References

1. Fuscoe FJ. *Med Lab Tech.* 1972; **29:** 261-271.

2. Russel FF. *J Med Res.* 1911; **25:** 217-229.

3. Kohn J. *J Path Bact.* 1954; **67:** 286-288.

4. Gillies RR. *J Clin Path.* 1956; **9:** 368-371.

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