New Media for the Differentiation of Enteric Bacteria

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Although a number of methods have been devised for rapid identification of colonies of enteric bacteria which appear on plates of differential media inoculated with fecal material, further improvement of these methods is needed. The two media described here have been used successfully by the author, and it is believed that their adoption will result in more rapid recognition of the pathogenic genera and in economy of time, materials, and labor. The compositions and methods of preparation of the media are as follows:

"IM" Medium

A. Basic medium:

Proteose peptone (Difco)	10.0 gm.
Sodium chloride	5.0 gm.
IM indicator	10.0 ml.
Distilled water to make	1,000.0 ml.

IM indicator is prepared by adding 0.4 gm. of thymol blue to 100 ml. of Andrade's indicator. The peptone and salt are dissolved in the distilled water, the reaction adjusted to pH 7.4, and the indicator added. The medium is distributed in 3-ml. amounts into 100 x 13 mm. test tubes which contain Durham inserts, and the tubes sterilized for 15 minutes at 15 pounds' pressure. Care should be taken that all air is expelled from the Durham tubes.

B. Triple carbohydrate solution:

Lactose	30.0 gm.
Mannitol	1.4 gm.
Sucrose	1.4 gm.
Distilled water to make	165.0 ml.

Substances of the highest purity obtainable should be used. After the materials are dissolved, the solution is sterilized by filtration, and stored in well-stoppered tubes in the refrigerator.

C. Urea solution:

A 20-percent urea solution is sterilized by filtration, distributed in stoppered tubes and stored in the refrigerator.

Add 5.0 ml. of urea solution to 8.2 ml. of carbohydrate solution and pipette 0.4 ml. of the mixture into each tube which contains 3.0 ml. of the basic medium. Incubate for 24 hours to insure sterility.

"SMG" Medium

A. Basic medium:

Proteose peptone (Difco)	5.0 gm.
Tryptone (Difco)	15.0 gm.
Lead acetate (neutral, C. P.)	0.5 gm.
Sodium thiosulfate (crystals, C. P.)	0.2 gm.
Agar (Bacto, Difco)	14.0 gm.
Phenol red (0.2 percent solution)	15.0 ml.
Distilled water to make	1,000.0 ml.

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Dissolve the peptone, tryptone, and agar in a boiling water bath. Dissolve the lead acetate and the sodium thiosulfate in a small amount of distilled water and add to the peptone-tryptone-agar solution. Adjust the medium to pH 7.4 to 7.5 and add the indicator. Distribute in 100- or 200-ml. amounts in suitable flasks and sterilize for 15 minutes at 15 pounds' pressure.

B. Double carbohydrate solution:

Mannitol	20.0 gm.
Glucose	2.0 gm.
Distilled water to make	110.0 ml.

Dissolve the carbohydrates and sterilize by filtration. Distribute aseptically in well-stoppered tubes in amounts of 5.5 or 11.0 ml.

To 100 ml. of melted and cooled basic medium add 5.5 ml. of the double carbohydrate solution, mix well, and distribute aseptically into 100 x 13 mm. tubes in amounts of 3.0 to 3.5 ml. Allow to solidify in a slanting position so that a deep butt is present. Incubate 24 hours to insure sterility.

In IM broth, urea utilization is indicated by production of alkali and consequent development of a blue color due to the presence of thymol blue. Acid production results in the development of a red color by the Andrade's indicator. The reading of the results in SMG

Biochemical reactions

Type of culture	IM broth			SMG agar			Remarks
	Urea	Acid	Gas	H_2S	Acid	Gas	
Shigella:							
Mannitol fermenters	−	+	−	−	+	−	In some strains, acidity vanishes in IM broth; in others (*S. sonnei*) it reappears very late.
Nonmannitol fermenters	−	−	−	−	−/+	−	
Salmonella:							
H_2S positive	−	−	+	++	+	++	
H_2S negative	−	−	+	−	+	++	Sometimes produce small amounts of H_2S.
Anaerogenic	−	+	−	−/++	+	−	
Paracolon (*Shigella*-like):							
29911 strains	−	−	−	−	−/+	−	Some strains produce small amounts of gas in SMG medium.
Other cultures	−	+	−	−	−/+	−	Do not ferment mannitol; but do ferment lactose or sucrose.
Paracolon:							
Salmonella-like (Arizona, Ballerup and Bethesda).	−	−	+	++	+	++	Some strains acidify IM very slowly; others produce very little H_2S.
Escherichia-like	−	±	+	−	+	++	Usually indol positive.
Anaerogenic	−	++	−	−	+	−	Ferment lactose.
Urea positive (produce alkali).	+	−	+	−	+	++	Very rare cultures.
Alcaligenes	−	−	−	−	−	−	
Pseudomonas	−	−	−	−	−	−	Pigment is best observed in IM medium.
Proteus:							
Nonmannitol fermenters:							
P. vulgaris	+	−	−	+	−/+	±	Sometimes produce little H_2S.
P. mirabilis							
P. morganii	±	−	−	±	−/+	±	Sometimes produce little H_2S.
Mannitol fermenters:							
P. rettgerii	+	−	−	−	++	−	
Escherichia:							
H_2S positive	−	+	++	++	+	++	
H_2S negative	−	+	++	−	+	++	
Aerobacter	−	−	++	−	+	++	

Key to the symbols:
− negative.
+ positive.
± late, very weak, or may even fail to appear.
−/+ neutral or alkaline slant, acid butt.
++ strong or very strongly positive.
−/++ negative to strongly positive.

agar is similar to the recording of reactions in Kligler's iron agar.

The media are inoculated with a thin platinum wire which is bent at an obtuse angle 1.5 to 2.0 mm. from its lower end. Each suspected colony is subcultured to IM broth, using a liberal inoculum which should be well distributed throughout the tube. The wire is sterilized and dipped into the inoculated broth, smeared on the surface of the SMG agar slant, and stabbed to the bottom of the tube. The tubes are incubated overnight at 37° C. The reactions of various groups of enteric bacteria in the two media are given in the accompanying table.

By incubating the tubes for several days, delayed fermentation of lactose or sucrose may become apparent. Often it may be desirable to test the action of the organisms on lactose, sucrose, adonitol, and salicin in the conventional manner. With many cultures, tests for indol, acetyl-methyl carbinol, citrate utilization, and motility will be necessary to confirm the tentative diagnosis.

The following notes may be helpful in the interpretation of the reactions:

1. Cultures which produce a strong acid reaction with abundant gas formation in IM broth and which do not blacken SMG agar are *Escherichia.*

2. When the amount of gas in IM medium is more than a small bubble, acid production is not apparent, and the turbidity is greater than usual; when a heavy growth is present in SMG medium, the inoculated organisms are not *Salmonella* or *Shigella* but are paracolon, *Escherichia*, or *Klebsiella.*

3. Cultures which produce a strong alkalinity in IM medium are *Proteus.* Reactions of different species are given in the table.

4. *Salmonella paratyphi A* and *Salmonella choleraesuis* do not blacken SMG agar.

5. Mannitol fermenting *Shigella* forms and anaerogenic *Salmonella* cultures produce similar reactions in the media and should be distinguished by motility and agglutination tests.

6. *Shigella* cultures which do not ferment mannitol produce no change in IM medium. *Shigella*-like paracolon strains and some *Proteus morgani* cultures give similar reactions but their greater growth vigor and characteristic odor aid in differentiation.

Growth from the surface of SMG agar may be suspended in saline and used as antigen in slide agglutination tests. Polyvalent *Shigella* serums, polyvalent *Salmonella* serums, and grouping serums for the two genera should be used to establish a rapid diagnosis of pathogenic forms which may be present.

Summary

Two media, IM broth and SMG agar, are described. By simultaneous inoculation of these media with colonies from plates inoculated with fecal material, it is possible to establish a rapid diagnosis of the enteric bacteria present. By their use it is possible to determine fermentation of glucose, mannitol, lactose, and sucrose; gas production, urea utilization; and H_2S production in two tubes. Results should be confirmed by appropriate biochemical and serological tests.

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