Technical Information

Hemmes Medium Base

Product Code: DM 1775

Application: Hemmes Medium Base is a screening medium for *Salmonella* and *Shigella* . The differentiation is based on seven reactions namely - dextrose, lactose, and sucrose fermentation, hydrogen sulphide production, urease detection, indole production and motility testing.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	3.000
Dextrose	0.300
Lactose	10.000
Sucrose	10.000
Sodium chloride	4.000
Ferrous sulphate	0.040
Sodium thiosulphate	0.100
Phenol red	0.015
Agar	5.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Hemmes Medium is used for screening and differentiating *Salmonella* and *Shigella.* The differentiation is based on seven reactions namely-dextrose, lactose, and sucrose fermentation, hydrogen sulphide production, urease detection, indole production and motility testing. Thus it is also named as Hemmes-7 Medium Base. It is prepared according to the formulation of Hemmes [3]. *Salmonella* and *Shigella* are gram-negative, facultatively anaerobic, non-sporulating, non-motile rods in the family *Enterobacteriaceae* [1, 2]. They are widely distributed in animals affecting mainly the stomach and the intestines. Arizona group was originally named *Salmonella* Arizonae. It has been found mainly in reptiles and birds and rarely in human patients with diarrhea or septicemia. These organisms are difficult to differentiate biochemically from *Escherichia coli. Salmonella* and *Shigella* show a red slant and a yellow butt from dextrose fermentation. Motility and gas formation are detectable because of the appropriate agar concentration. Indole formation is also detectable due to the presence of case in enzymic hydrolysate. Urease activity is detected by the formation of cerise colour. For the inoculation of this medium, pick isolated colonies from plates and streak the slant and stab the butt. Incubate at 37°C overnight. Casein enzymic hydrolysate and yeast extract in the medium are sources of carbon, nitrogen, vitamins and minerals. Ferrous sulphate, Sodium thiosulpahte and sodium chloride provide the essential ions. Dextrose, lactose, and sucrose are included in the medium for fermentation studies.

Methodology

Suspend 42.95 grams of powder media in 950 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense 95 ml amounts into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to about 50-55°C and aseptically add 5 ml of sterile 40% Urea Solution (MS2048) per 95 ml basal medium. Mix well and dispense into sterile test tubes. Allow the tubed medium to cool and solidify in the slanted position to give a butt of at least 3 cm and slant of 2 cm.

Quality Control

Physical Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.55% Agar gel.

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 4.3%w/v aqueous solution at 25°C. pH : 7.2±0.2

pH range 7.00-7.40

Cultural Response/Characteristics

DM 1775: Cultural characteristics observed with added 40% Urea solution (MS2048), after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	H_2S	Indole	Motility	Urease
Escherichia coli ATCC 25922	50-100	Luxuriant	negative reaction,no blackening of medium	Positive reaction red ring at the interface of the medium	positive, growth away from stabline causing turbidity variable, motility is temp dependent.	negative reaction, yellow slant
Proteus mirabilis ATCC 25933	50-100	Luxuriant	positive reaction, blackening of medium	Negative reaction no colour development/cloudly ring	It is more pronounced at 20°C and almost absent at 35°C	positive reaction pink colour throughout
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	Positive reaction blackening of medium	Negative reaction no colour development/cloudly ring	positive, growth away from stabline causing turbidity	negative reaction, yellow slant
Staphylococcus aureus ATCC 25923	50-100	luxuriant	Negative reaction,no blackening of medium	Negative reaction no colour development/cloudly ring	negative, growth along the stabline, surrounding medium remains clear	negative reaction, yellow slant

Further Reading

1. Ryan K. J., Ray C. G., (Eds.), 2004, Sherris Medical Microbiology, 4th Ed., McGraw Hill.
2. Giannella R. A., 1996, Salmonella In: Barons Medical Microbiology (Baron S. et al, Eds.), 4th Ed., Univ. of Texas, Medical Branch.
3. Hemmes J. H., St. Inst. Publ. Hlth., Curacao, Netherlands, Antilles.

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