Hemmes Medium Base

M775

Intended Use:

Recommended for biochemical differentiation of *Salmonella* and *Shigella* species based on dextrose, lactose, sucrose fermentation, motility, hydrogen sulphide, indole and urease production.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	3.000
Dextrose (Glucose)	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

Directions

Suspend 42.95 grams in 950 ml purified / distilled water. Heat to boiling 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 (FD048) 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.

Principle And Interpretation

Salmonella and *Shigella* are gram-negative, facultatively anaerobic, non-sporulating, non-motile rods in the family *Enterobacteriaceae* (1,5). 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 occasionally in human patients with diarrhea or septicemia. These organisms are difficult to differentiate biochemically from *Escherichia coli*. 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 (2).

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 tryptone. Urease activity is detected by the formation of cerise colour.

Tryptone 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.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

For the inoculation of this medium, pick isolated colonies from plates and streak the slant and stab the butt. Incubate at 37°C overnight. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Well isolated pure colonies must be used.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.55% Agar gel.

Colour and Clarity

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

7.00-7.40

Cultural Response

Cultural characteristics observed with added 40% Urea solution (FD048), after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	H2S	Indole	Motility	Urease
Escherichia coli ATCC 25922 (00013*)	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	negative reaction, yellow slant
Proteus mirabilis ATCC 25933	50-100	luxuriant	positive reaction, blackening of medium	negative reaction, no colour development / cloudy ring	variable, motility is temp. dependent. It is more pronounced at 20°C and almost absent at 35°C	positive reaction, pink colour throughout
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	positive reaction, blackening of medium	negative reaction, no colour development / cloudy ring	positive, growth away from stabline causing turbidity	negative reaction, yellow slant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	negative reaction,no blackening of medium	negative reaction, no colour development / cloudy ring	negative, growth along the stabline, surrounding medium remains clear	negative reaction, yellow slant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with

sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Giannella R. A., 1996, Salmonella In: Barons Medical Microbiology (Baron S. et al, Eds.), 4th Ed., Univ. of Texas, Medical Branch.
2. Hemmes J. H., St. Inst. Publ. Hlth., Curacao, Netherlands, Antilles.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Ryan K. J., Ray C. G., (Eds.), 2004, Sherris Medical Microbiology, 4th Ed., McGraw Hill.

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