Urea Broth Medium 18 (In accordance with IP 2007)

MM111

Intended Use:

Recommended for identification of bacteria on the basis of urea utilization, specifically for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in accordance with IP.

Composition**

Ingredients	Gms / Litre
Potassium dihydrogen orthophosphate	9.100
Yeast extract	0.100
Anhydrous disodium hydrogen phosphate	9.500
Urea	20.000
Phenol red	0.010

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.71 grams in 1000 ml purified/distilled water. Mix thoroughly to dissolve the medium completely. Sterilize by filtration. Aseptically dispense in sterile test tubes or flasks as desired.

Note : Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. User must ensure its compatibility with the latest edition.

Principle And Interpretation

Urea Broth Medium was developed by Rustigian and Stuart (1). This medium is especially recommended by Indian Pharmacopoeia (2) for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in the enteric infection diagnosis (3), based on urea utilization (4,5). It is also recommended for microbial limit tests. Other Gram-negative enteric bacilli are unable to utilize urea and fails to grow because of reduced availability of other nutrients. Urea Broth Medium becomes alkaline as the utilization of urea by the organisms liberate ammonia during the incubation, indicated by pink red colour. All urea test media rely on the alkalinity formation and so they are not specific for urease testing. Yeast extract provides essential vitamins and other growth factors. Phosphates aids as good buffering agent. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids results in false-positive reaction. This medium shows positive reaction with Genus *Proteus*, few *Providencia* and *Morganella* sp. species.

Type of specimen

Pure isolate.

Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (2) . 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. Prolonged incubation may cause alkaline reaction in the medium.
2. Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (7).

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 light pink homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow orange coloured clear solution

Growth Promotion Test
Growth Promotion is carried out in accordance with Indian Pharmacopoeia

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Urease
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	Negative reaction,no change
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	Negative reaction, no change
Proteus vulgaris ATCC 13315	50-100	Positive reaction, cerise colour
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	Positive reaction, cerise colour
Escherichia coli ATCC 8739 (00012*)	50-100	Negative reaction,no change
Klebsiella pneumoniae ATCC 10031	50-100	Positive reaction, cerise colour
Escherichia coli NCTC 9002	50-100	Negative reaction,no change

Key : *Corresponding WDCM numbers.#- Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store dehydrated 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 (6,7).

Reference

1.Rustigian and Stuart, 1941, Proc. Soc. Exp. Biol. Med., 47:108.
2.Indian Pharmacopoeia, 2007 Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
3.Forbes, B.A.; Sahm, D.F. and Weissfelf, A.S., 2002, Bailey and Scott s Diagnostic Microbiology, 11th ed., The C.V. Mosby Co., St. Louis.
4.MacFaddin J., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd ed.,Williams and Wilkins, Baltimore.
5.Christensen, 1946, J. Bact., 52:461.
6.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7.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.

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