



## Urea Broth Base (Diagnostic Stuarts Urea Broth Base)

M111

### Intended Use:

Urea Broth is recommended for the identification of bacteria on the basis of urea utilization, specifically for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species.

### Composition\*\*

Ingredients	Gms / Litre
Monopotassium phosphate	9.100
Dipotassium phosphate	9.500
Yeast extract	0.100
Phenol red	0.010
Final pH ( at 25°C)	6.8±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 18.71 grams in 950 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 55°C. Aseptically add 50 ml of sterile 40% Urea solution (FD048). Mix well and distribute in 10 ml amounts into sterile tubes.

### Principle And Interpretation

Rustigian and Stuart developed Urea Broth (8). This medium is especially recommended for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in the enteric infection diagnosis (4), based on urea utilization (3, 7). Gram-negative enteric bacilli are unable to utilize urea because of less nutrients and high buffering capacity of the medium. Urea Broth becomes alkaline as the utilization of urea by the organisms liberates 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. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids results in false-positive reaction. A medium without urea serves as negative control to rule out false positive results.

### Type of specimen

Pure isolate.

### Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,4,9).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

In Vitro diagnostic use only. 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 clinical 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

Yellowish orange coloured clear solution in tubes.

#### Reaction

Reaction of basal medium (1.87gm in 95ml distilled water) at 25°C. pH : 6.8±0.2

#### pH

6.60-7.00

#### Cultural Response

Cultural characteristics observed on addition of sterile 40% Urea solution (FD048) after an incubation at 35-37°C for 18-24 hours.

#### Cultural Response

Organism	Inoculum (CFU)	Urease
<b>Cultural Response</b>		
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	negative reaction, no change
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	negative reaction, no change
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	positive reaction, cerise colour
<i>Escherichia coli</i> NCTC 9002	50-100	negative reaction, no change
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	negative reaction, no change
<i>Salmonella Typhimurium</i> ATCC 14028 (00031*)	50-100	negative reaction, no change
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	positive reaction, cerise colour
<i>Proteus vulgaris</i> ATCC 13315	50-100	positive reaction, cerise colour
<i>Proteus mirabilis</i> ATCC 25933	50-100	positive reaction, cerise colour

Key : \*Corresponding WDCM numbers.

#- Formerly known as *Enterobacter aerogenes*

### Storage and Shelf Life

Store below 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. Use before expiry date on the label. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

## Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Christensen, 1946, J. Bact., 52:461.
4. Finegold and Baron, 1986, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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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9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

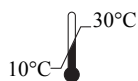
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