Urea Broth Base

Intended Use
Urea Broth Base is a medium with added urea, used for the detection of urease production, to differentiate *Proteus* species from *Salmonella* and *Shigella* species.

Summary
Rustigian and Stuart developed Urea Broth Base for the identification of bacteria on the basis of urea utilization and is particularly recommended for the differentiation of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections. The broth is positive for *Proteus*, *Morganella morganii* subspecies *morganii*, *Providencia rettgeri* and a few *Providencia stuartii* strains with the reclassification of the members of the genus Proteeae. Urea Broth Base is included in the Bacteriological Analytical Manual for food and cosmetics testing, in IP and is recommended by APHA in the examination of milk and foods.

Principle
Yeast extract provides trace elements, vitamins and amino acids. 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 utilization of urea by the organisms liberates ammonia during incubation, which is indicated by pink red colour. Since this test relies on the alkalinity formation, it is 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.

Formula*

Ingredients	**g/L**
Yeast Extract	0.1
Dipotassium Phosphate	9.5
Monopotassium Phosphate	9.1
Phenol Red	0.01
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 18.71 g of the powder in 950 mL purified / distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 55°C.
5. Aseptically add 50 mL of sterile 40% Urea Solution (204210060005) and distribute10 mL aliquots into sterile tubes.

Quality Control
Dehydrated Appearance: Light orange coloured, homogenous, free flowing powder.
Prepared Appearance: Yellow orange coloured, clear solution without any precipitate.
Cultural Response: Cultural characteristics observed after an incubation of 18-48 hours at 30°C-35°C.

Organism (ATCC)	Growth	Urease
Klebsiella aerogenes (13048)	Good	-
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	-
Escherichia coli (25922)	Good	-
Proteus mirabilis (25933)	Good	+

Key:

+ = Positive, pink - red colour

- = Negative, no change

Interpretation of Results

1. The production of urease is indicated by an intense pink-red colour throughout the broth.
2. A negative reaction is no colour change. The broth medium remains pale yellow to buff.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. It is preferable that the medium be used on the day of preparation. If not, examine the tubes carefully to ensure sterility.
2. Do not reheat the medium after the addition of Urea 40% as urea decomposes very easily.
3. To rule out false-positives due to protein hydrolysis (as opposed to urea hydrolysis) that may occur in the medium after prolonged incubation, perform a control test without urea.
4. The high buffering system in this medium, masks urease activity in organisms that are delayed positive. This medium is therefore recommended for the detection of urease activity in all *Proteus* species. *Providencia rettgeri* and urease-positive *Providencia stuarlii*, *M. morganii* slowly hydrolyze urea and may require approximately a 36-hour incubation for strong urease positive reaction to occur. When in doubt as to a result, compare with an un-inoculated tube or incubate for an additional 24 hours.
5. Variations in the size of the inoculum can affect the time required to reach positive results.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Christensen WB; 1946, J. Bact; Vol. 52:461.
2. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
3. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol. 2.
4. Downes and Ito (ed.) 2001, Compendium of Methods for The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
5. Rustigian and Stuart, 1941, Proc. Soc. Exp. Bio. Med; 47: 108.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product Description	Pack Size
201210030100	Dehydrated Culture Media	100 g
201210030500	Dehydrated Culture Media	500 g
203210040012	Ready Prepared Slant	12 Slants