BBL™ Urea Agar Base Concentrate 10X
BBL™ Urea Agar Slants, Complete

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QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Urea Agar is a differential medium for members of the *Enterobacteriaceae* on the basis of their ability to produce urease.

II PERFORMANCE TEST PROCEDURE

A. Directions for preparation of a complete medium from Urea Agar Base Concentrate 10X

1. To prepare Urea Agar medium, add 1.7 g of granulated agar to 100 mL of purified water. Heat with agitation and boil for 1 min.
2. Dispense in 9 mL aliquots into tubes and sterilize by autoclaving at 121 °C for 15 min.
3. Cool the agar to 45–50 °C, and allow one tube of concentrate to come to room temperature. Add 1 mL of concentrate to each 9 mL of cooled agar solution and mix thoroughly.
4. Allow the tubes to cool in a slanted position so that slants with deep butts are formed.

B. Testing of complete medium (Urea Agar Slants)

1. Inoculate representative samples with the cultures listed below.
   a. Using a 0.01 mL calibrated loop, inoculate the slant surfaces with heavy inocula using 24- to 48-h **Trypticase™** Soy Agar Slant cultures. Do not inoculate the butt.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 2, 4, 6 and 24 h for growth and reactions.
3. Expected Results

Organisms	ATCC®	Urease Reaction
**Proteus vulgaris*	8427	+ (Intense pink-red to red-violet color)
Morganella morganii subsp. *morganii*	8019	+ (Intense pink-red to red-violet color)
**Salmonella enterica* subsp. *enterica* serotype Typhimurium	13311	– (No color change)

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Urea Agar is used for the differentiation of organisms, especially the *Enterobacteriaceae*, on the basis of urease production.

V SUMMARY AND EXPLANATION

Urea Agar was devised by Christensen for use as a solid medium for the differentiation of enteric bacilli.[1] It differentiates between rapid urease-positive *Proteeae* organisms (*Proteus* spp., *Morganella morganii* subsp. *morganii*, *Providencia rettgeri*, and some *Providencia stuartii*) and other urease-positive organisms: *Citrobacter*, *Enterobacter* and *Klebsiella* and bacteria other than *Enterobacteriaceae*; i.e., some *Bordetella* and *Brucella* species.[2]

The base is also supplied as a filter-sterilized 10X concentrated solution in tubes for use in preparing Urea Agar slants in the user’s laboratory.

VI PRINCIPLES OF THE PROCEDURE

The urea medium of Rustigian and Stuart[3] is particularly suited for the differentiation of *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea;[1] the latter are unable to do so in Urease Test Broth because of limited nutrients and the high buffering capacity of the medium. To provide a medium with greater utility, Urea Agar was devised by Christensen[1] with peptone and dextrose included and reduced buffer content to promote more rapid growth of many of the *Enterobacteriaceae* and permit a reduction in incubation time.

When organisms utilize urea, ammonia is formed during incubation which makes the reaction of these media alkaline, producing a pink-red color. Consequently, urease production may be detected by the change in the phenol red indicator.

VII REAGENTS

Urea Agar Base Concentrate 10X

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	10.0 g	Potassium Phosphate	20.0 g
Dextrose	10.0 g	Urea	200.0 g
Sodium Chloride	50.0 g	Phenol Red	0.12 g

*Adjusted and/or supplemented as required to meet performance criteria.

Urea Agar Slants, Complete

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	1.0 g	Urea	20.0 g
Dextrose	1.0 g	Phenol Red	0.012 g
Sodium Chloride	5.0 g	Agar	15.0 g
Potassium Phosphate	2.0 g		

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. Prior to discarding, sterilize prepared tubes, specimen containers and other contaminated materials by autoclaving.

221100 BD BBL™ Urea Agar Base Concentrate 10X, Pkg. of 10 size K tubes

Warning

H315 Causes skin irritation. **H319** Causes serious eye irritation.
P103 Read label before use. **P264** Wash thoroughly after handling. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Minimize exposure to light.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[4,5] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Urea Agar Base Concentrate 10X or Urea Agar Slants, Complete

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

If the Urea Agar Base Concentrate 10X is being used, prepare the complete medium as described in the Quality Control section. If crystals form in the concentrate, they will usually dissolve at room temperature, or in a few minutes in a 40 °C water bath.

Using a heavy inoculum of growth from an 18- to 24-h pure culture (TSI Agar or other suitable medium), streak back and forth over the entire slant surface. Do not stab the butt since it serves as a color control. Incubate tubes with loosened caps at 35 ± 2 °C in an incubator or water bath. Observe reactions after 2, 4, 6 and 24 h and every day thereafter for a total of 6 days. Even longer incubation periods may be necessary.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

X RESULTS

The production of urease is indicated by an intense pink-red (red-violet) color on the slant. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis.[6]

A negative reaction is no color change; the agar medium remains pale yellow to buff.

For a listing of urease-positive organisms, consult appropriate texts.[5,7,8]

XI LIMITATIONS OF THE PROCEDURE

1. These urea test media rely on the demonstration of alkalinity; hence, they are not specific for urease. The utilization of peptones, especially in slant agar (e.g., by *Pseudomonas aeruginosa*), or other proteins in the medium may raise the pH to alkalinity due to protein hydrolysis and release of excessive amino acid residues, resulting in false-positive reactions.[2]
2. On Urea Agar, urease-positive *Proteeae* cause the medium to turn alkaline soon after inoculation. For the results to be valid for the detection of *Proteeae*, the results must be read within the first 6 h of incubation. *Citrobacter freundii* and *Klebsiella pneumoniae* subsp. *pneumoniae* may produce positive reactions within 24–48 h.[2]
3. For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[4,5,7]

XII PERFORMANCE CHARACTERISTICS

Kantor et al. developed a rapid and simplified scheme, using a minimum of three tests and a maximum of seven tests, that could be used routinely by laboratories to identify nonfermentative gram-negative bacteria. A total of 229 unknown nonfermentative gram-negative organisms and 14 reference strains were identified using this scheme. Urea Agar was used to differentiate *Alcaligenes* sp. and *Pseudomonas alcaligenes* from *Bordetella bronchiseptica*.[9]

XIII AVAILABILITY

Cat. No.	Description
221100	**BD BBL™** Urea Agar Base Concentrate 10X, Pkg. of 10 size K tubes
221096	**BD BBL™** Urea Agar Slants, Complete, Pkg. of 10 size K tubes
221097	**BD BBL™** Urea Agar Slants, Complete, Ctn. of 100 size K tubes

XIV REFERENCES

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3. Rustigian, R., and C.A. Stuart. 1941. Decomposition of urea by Proteus. Proc. Soc. Exp. Biol. Med. *47*:108-112.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
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6. Ewing, W.H. 1985. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York.
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8. Farmer, J.J., III. 1999. Enterobacteriaceae: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
9. Kantor, L.T., S.D. Kominos, and R.B. Yee. 1975. Identification of nonfermentative gram-negative bacteria in the clinical laboratory. Am. J. Med. Technol. *41*:3-9.

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