Urea Media
Urea Agar Base • Urea Agar Base Concentrate 10×
Urea Agar • Urea Broth • Urease Test Broth
Urease Broth Concentrate 10×

Intended Use

Urea Agar and Urease Test Broth are used for the differentiation of organisms, especially the *Enterobacteriaceae*, on the basis of urease production.

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* spp.[2]

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

Urease Test Broth was developed by Rustigian and Stuart.[3] It may be used for the identification of bacteria on the basis of urea utilization and it is particularly recommended for the differentiation of members of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections.[4] The medium is positive for *Proteus*, *Morganella morganii* subsp. *morganii*, *Providencia rettgeri*, and a few *Providencia stuartii* strains with the reclassification of the members of the *Proteeae*.

Urease base is also supplied as a filter sterilized 10× concentrated solution for use in preparing Urease Test Broth in the laboratory.

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. The complete Urea Agar contains 15.0 g/L of agar in addition to the ingredients in the base medium.

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

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Urea Broth

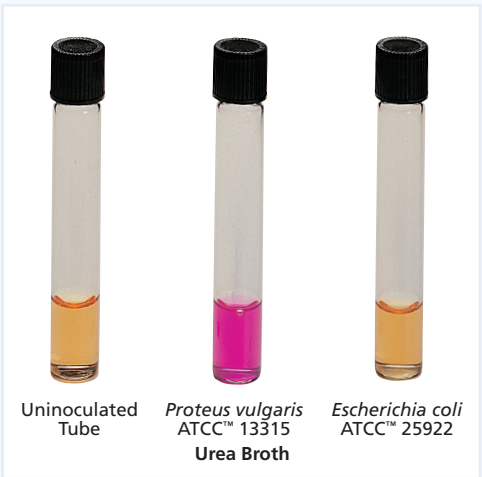
Dehydrated Appearance:	Light orange to light pink, homogeneous, inherently lumpy.
Solution:	3.87% solution, soluble in purified water. Solution is orange-yellow, clear.
Prepared Appearance:	Orange-yellow, clear.
Reaction of 3.87% Solution at 25°C:	pH 6.8 ± 0.1

Cultural Response

Difco™ Urea Broth

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 8-48 hours.

ORGANISM	ATCC™	UREASE REACTION
Enterobacter aerogenes	13048	–
Escherichia coli	25922	–
Proteus mirabilis	25933	+
Proteus vulgaris	13315	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	–



Uninoculated Tube | *Proteus vulgaris* ATCC™ 13315 | *Escherichia coli* ATCC™ 25922

Urea Broth

Identity Specifications

BBL™ Urea Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	29 g/100 mL solution, soluble in purified water. Complete medium is light to medium, orange, clear to slightly hazy.
Prepared Appearance:	Complete medium is light to medium, orange, clear to slightly hazy.
Reaction of 2.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Urea Agar Base

Prepare the medium per label directions. Inoculate with fresh cultures (2 heavy loopfuls) and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	UREASE REACTION
Proteus vulgaris	8427	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	13311	–

Uninoculated Tube | *Proteus vulgaris* ATCC™ 13315 | *Escherichia coli* ATCC™ 25922

Urea Agar

Formulae

BBL™ Urea Agar Base

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	1.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Potassium Phosphate	2.0	g
Urea	20.0	g
Phenol Red	12.0	mg

Difco™ Urea Broth

Approximate Formula* Per Liter

Yeast Extract	0.1	g
Monopotassium Phosphate	9.1	g
Dipotassium Phosphate	9.5	g
Urea	20.0	g
Phenol Red	0.01	g

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

Directions for Preparation from Dehydrated Product

BBL™ Urea Agar Base

1. Dissolve 29 g of the powder in 100 mL of purified water. Mix thoroughly. Sterilize by filtration.
2. Suspend 15 g of agar in 900 mL of purified water.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 100 mL of the sterile Urea Agar Base.
5. Mix thoroughly and dispense aseptically in sterile tubes.
6. Cool tubed medium in a slanted position so that deep butts are formed.
7. Do not remelt the complete medium.
8. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Urea Agar Base Concentrate 10× (Prepared Tubes)

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 minute.
2. Dispense in 9 mL aliquots into tubes and autoclave at 121°C for 15 minutes.
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.
5. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Urea Broth

1. Equilibrate the medium to room temperature before opening. The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.
2. Dissolve 38.7 g of the powder in 1 L of purified water. Mix thoroughly to completely dissolve the powder.
3. Filter sterilize. DO NOT BOIL OR AUTOCLAVE THE MEDIUM.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Urease Broth Concentrate 10× (Prepared Tubes)

1. To prepare medium, aseptically add 10 mL of the concentrate to 90 mL of cold sterile purified water. Mix thoroughly.
2. Dispense aseptically in 1-3 mL amounts, in small sterile test tubes.

Procedure

If Urea Agar Base Concentrate 10× or Urease Broth Concentrate 10× is being used, prepare the complete medium as described above. 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 (2 loopfuls) of growth from an 18- to 24-hour pure culture (TSI Agar or other suitable medium), inoculate the broth or agar (streaking back and forth over the entire slant surface). Do not stab the butt since it serves as a color control. For broth, shake tubes gently to suspend the bacteria. Incubate tubes with loosened caps at 35 ± 2°C in an incubator or water bath. Observe reactions after 2, 4, 6, 18, 24 and 48 hours. For agar, continue to check every day for a total of 6 days; even longer incubation periods may be necessary.

Expected Results

The production of urease is indicated by an intense pink-red (red-violet) color on the slant or throughout the broth. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis.[5]

A negative reaction is no color change. The agar medium remains pale yellow to buff; the broth remains yellowish-orange.

For a listing of urease-positive organisms, consult appropriate texts.[2, 4-7]

Limitations of the Procedure

Urea Agar Base

1. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (especially in slant agar by *Pseudomonas aeruginosa*, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.[7]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Urea Agar detects rapid urease activity of only the urease-positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within the first 2-6 hours after incubation. Urease-positive *Enterobacter*, *Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.

Urea Broth

1. 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 with the same test medium without urea.[7]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. 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* spp., *Providencia rettgeri* and urease-positive *Providencia stuartii*.[1] *M. morganii* slowly hydrolyzes urea and may require approximately a 36 hour incubation for a strong urease-positive reaction to occur.[1] If in doubt as to a result, compare with an uninoculated tube or incubate for an additional 24 hours.
4. Variations in the size of the inoculum can affect the time required to reach positive (alkaline, pH 8.1) results.

References

1. Christensen. 1946. J. Bacteriol. *52*:461.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Rustigian and Stuart. 1941. Proc. Soc. Exp. Biol. Med. *47*:108.
4. Ewing. 1985. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Urea Agar Base

BAM CCAM ISO USDA

Cat. No. 211795 Dehydrated – 500 g*

BBL™ Urea Agar Base Concentrate 10×

Cat. No. 221100 Prepared Tubes – Pkg. of 10*

BBL™ Urea Agar

Cat. No. 221096 Prepared Slants – Pkg. of 10*
221097 Prepared Slants – Ctn. of 100*

Difco™ Urea Broth

AOAC BAM COMPF SMD

Cat. No. 227210 Dehydrated – 500 g*

BBL™ Urease Test Broth

AOAC BAM COMPF SMD

Cat. No. 221719 Prepared Tubes, 3 mL – Pkg. of 10*

BBL™ Urease Broth Concentrate 10×

Cat. No. 221098 Prepared Tubes – Pkg. of 10*

**Store at 2-8°C.*