UREA MEDIUM

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]

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*.

PRINCIPLE

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.

REAGENTS (FORMULA)

Yeast Extract	0.1	g
Dipotassium phosphate	9.5	g
Monopotassium Phosphate	9.1	g
Urea	20.0	g
Phenol Red	0.01	g
Agar	15.0	g
Deionized Water	1000.0	ml

PROCEDURE

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, 4-5]

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Proteus vulgaris ATCC 13315	35 ± 2°C for 8-48 hours	Urease (+)
Escherichia coli ATCC 25922	35 ± 2°C for 8-48 hours	Urease (-)

BIBLIOGRAPHY

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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.

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