A-8 AGAR SELECTIVE and NONSELECTIVE

INTENDED USE
Remel A-8 Agar, Selective and Nonselective, are solid media recommended for use in qualitative procedures for the cultivation, identification, and differentiation of *Ureaplasma urealyticum* and *Mycoplasma hominis.*

SUMMARY AND EXPLANATION
The discovery of a urease enzyme system in *U. urealyticum* made methods for identification of ureaplasmas possible.[1] Urease enzymes hydrolyze urea with the production of ammonia and resultant alkalinity. A differential medium for *Ureaplasma* (A-7 Agar) was developed by Shepard and Lunceford which contained urea with manganous sulfate as an indicator. The ammonia produced during urea hydrolysis results in the precipitation of a metallic oxide reaction product within and on the surface of *Ureaplasma* colonies.[2] In 1979, Shepard and Combs added putrescine dihydrochloride to A-7 differential medium to enhance colony size. This resulted in a significant increase in the mean colony size of *Ureaplasma* isolates and intensified visualization of ammonia diffusion in the agar surrounding the colonies.[3,4]

PRINCIPLE
Casein and soy peptones supply nitrogenous substances and amino acids necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Dipotassium phosphate provides a buffering action. Dextrose is added as an energy source. L-cysteine hydrochloride is a reducing agent which minimizes the formation of inhibitory substances. Normal horse serum is added as a source of sterols. Yeast extract supplies B-complex vitamins and serves as a growth enhancer. Putrescine is a polyamine, which enhances growth and intensifies ammonia diffusion in the agar. Urea is required for the growth of ureaplasmas. GHL triptide is a liver cell growth factor and calcium chloride is a precipitating agent. Cefoperazone is a broad-spectrum antibiotic which inhibits many gram-negative and gram-positive organisms; amphotericin B inhibits yeast and molds.

REAGENTS (CLASSICAL FORMULA)*
Casein Peptone .... 17.0 g
Sodium Chloride .... 5.0 g
Soy Peptone .... 3.0 g
Dextrose .... 2.5 g
Dipotassium Phosphate .... 2.5 g
Putrescine Dihydrochloride .... 1.7 g
Calcium Chloride .... 0.5 g
GHL Tripeptide .... 0.02 mg
Normal Horse Serum .... 200.0 ml
Urea 10% .... 10.0 ml
Yeast Extract .... 10.0 ml
L-Cysteine Hydrochloride .... 1.0 ml
Agar .... 11.8 g
Demineralized Water .... 770.0 ml

pH 6.0 +/- 0.2 @ 25°C

The following ingredients are added per liter of A-8 Selective Agar:
Amphotericin B .... 2.5 mg
Cefoperazone .... 20.0 mg

The following ingredient is added per liter of A-8 Nonselective Agar:
Penicillin G .... 1,000,000 U

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prepare a 1:10 dilution of the specimen in a suitable transport medium, such as 10B Broth (REF 20302) or TSB w/ 0.5 Bovine Albumin (REF 065006), following recommended guidelines.
2. Using a sterile pipette, transfer an aliquot from the broth to the A-8 Agar, Selective or Nonselective.
3. Streak the plate for isolation and seal closed to restrict dehydration.
4. Incubate the plate in 5% CO_2 or in an anaerobic atmosphere at 35-37°C.
5. Examine microscopically for growth and typical colonial morphology for up to 7 days. *U. urealyticum* produces golden brown colonies on A-8 Agar. Colonies of *M. hominis* may exhibit a typical "fried egg" appearance.[4,5]

NOTE: Serial dilutions to 10^{-3} of any specimen will optimize recovery and is recommended to overcome potential inhibitory substances that may be present in the medium or in the specimen.[3,4]

QUALITY CONTROL
All lot numbers A-8 Agar, Selective and Nonselective, have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
A-8 Nonselective:		
Ureaplasma urealyticum ATCC® 27618	CO_2, 72 h @ 33-37°C	Good growth, golden brown colonies
Mycoplasma hominis ATCC® 23114	CO_2, 72 h @ 33-37°C	Good growth
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
A-8 Selective:		
Ureaplasma urealyticum ATCC® 27618	CO_2, 72 h @ 33-37°C	Good growth, golden brown colonies
Mycoplasma hominis ATCC® 23114	CO_2, 72 h @ 33-37°C	Good growth
Candida albicans ATCC®10231	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

(Continued on back)

LIMITATIONS

1. Ureaplasmas are extremely susceptible to a rapid, steep death phase in culture due to urea depletion and elevated pH and must be monitored and subcultured to appropriate media on a regular basis to obtain maximum viability.[5]

BIBLIOGRAPHY

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4. Winn, W.C., S.D. Allen, W.M. Janda, E.W. Koneman, G.W. Procop, P.C. Schreckenberger, and G.L. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol.1. Williams & Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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