A-7 AGAR

INTENDED USE
Remel A-7 Agar is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Ureaplasma* and some species of *Mycoplasma*.

SUMMARY AND EXPLANATION
A-7 Agar was first described in 1976 by Shepard and Lunceford.[1] This medium incorporates the principle of the urease spot test in a culture medium. A-7 Agar contains added urea and manganous sulfate, a sensitive indicator of ammonia. *Ureaplasma urealyticum* produces an enzyme which hydrolyzes urea with the production of ammonia and resultant alkalinity. A-7 Agar also supports the growth of *Mycoplasma hominis* which produces characteristic colonies with a typical fried-egg appearance.[2,3]

PRINCIPLE
Casein and soy peptones supply nitrogenous compounds 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 an energy source. L-cysteine hydrochloride is a reducing agent which minimizes inhibitory substances. Horse serum and GCHI are added as enrichments. Yeast extract supplies B-complex vitamins and serves as a growth enhancer. Manganous sulfate detects ammonia produced when urea is hydrolyzed by *Ureaplasma*. Urease-positive colonies turn brown due to the formation of particles of manganese dioxide. Amphotericin B and penicillin are selective agents which inhibit gram-positive cocci and some fungi.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	17.0	g	Normal Horse Serum	200.0	ml
Sodium Chloride	5.0	g	●GCHI	10.0	ml
Soy Peptone	3.0	g	Urea 10%	10.0	ml
Dextrose	2.5	g	Yeast Extract	10.0	ml
Dipotassium Phosphate	2.5	g	L-Cysteine Hydrochloride	1.0	ml
Manganous Sulfate	0.15	g	Penicillin G	1,000,000	U
Amphotericin B	2.5	mg	Agar	11.8	g
			Demineralized Water	770.0	ml

pH 6.0 ± 0.2 @ 25°C

●GCHI Enrichment:

Glucose	100.0	g	Cocarboxylase	0.1	g
Cysteine Hydrochloride	25.9	g	Guanine Hydrochloride	0.03	g
L-Glutamine	10.0	g	Ferric Nitrate	0.02	g
L-Cystine	1.1	g	Vitamin B-12	0.01	g
Adenine	1.0	g	P-Aminobenzoic Acid	13.0	mg
NAD	0.25	g	Thiamine Hydrochloride	3.0	mg
			Demineralized Water	1000.0	ml

*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-7 Agar.
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 intensely dark, golden brown colonies on A-7 Agar. Colonies of *Mycoplasma hominis* may exhibit a typical "fried egg" appearance.[3]

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.[2,3]

QUALITY CONTROL
All lot numbers of A-7 Agar 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
Ureaplasma urealyticum ATCC® 27618	CO_2, 72 h @ 33-37°C	Growth, golden brown colonies
Mycoplasma hominis ATCC® 23114	CO_2, 72 h @ 33-37°C	Growth
Candida albicans ATCC® 10231	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)

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.[4]

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BIBLIOGRAPHY

1. Shepard, M.C. and C.D. Lunceford. 1976. J. Clin. Microbiol. 3:613-625.
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4. 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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