



Instructions for Use

A8 AGAR

Cat. no. G02	A8 Agar, 15x60mm Plate, 11ml	10 plates/bag
------------------------------	------------------------------	---------------

INTENDED USE

Hardy Diagnostics A8 Agar is used for isolation and differentiation of genital mycoplasmas, i.e. *Ureaplasma urealyticum* and *Mycoplasma hominis*.

SUMMARY

A8 Agar is highly nutritious due to peptones supplemented with yeast extract and inactivated horse serum present in the medium. The yeast extract provides diphosphopyridine nucleotides and the serum provides cholesterol and a source of protein. Amphotericin B and penicillin G are added to inhibit faster growing contaminants.

The discovery of a urease enzyme system in *Ureaplasma urealyticum* makes methods of identification possible. *Ureaplasma urealyticum* can be differentiated from other genital mycoplasmas on A8 due to manganous sulfate in the medium which combines with the urease to form a golden brown pigment.

FORMULA

Ingredients per 766ml of deionized water:*

Putrescine, 2HCl	34.0gm
Tryptic Soy Broth	24.0gm
Dipotassium Phosphate	2.5gm
Dextrose	2.5gm
Manganous Sulfate	0.2gm
L-Cysteine, HCl	0.1gm
Amphotericin B	2.5mg
GHL Tripeptide	2.0mg
Horse Serum	188.0ml
Yeast Extract	35.0ml
Urea Solution, 10%	10.0ml
Koenzyme Enrichments	1.0ml
Penicillin G	1,000,000U

Agar

11.0gm

Final pH 6.0 +/- 0.2 at 25°C

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Specimen/swab should be placed in a tightly sealed transport container with suitable transport medium to prevent drying and taken directly to the laboratory. Dacron, polyester, or calcium alginate swabs are acceptable. Samples should be processed as soon as possible after arrival to the laboratory. If there is to be a delay in culturing, specimens should be refrigerated at 2-8°C. For long-term storage, or if specimens cannot be cultured within 24 hours, freeze specimens at -70°C. Do not freeze at temperatures greater than -70°C.

Method of Use: Vaginal swabs, urethral swabs, cervical swabs and urine are suitable for culturing. Urethral exudates are streaked with a loop or rolled with a swab on culture plates immediately. Urine specimens are centrifuged, the supernatant removed, and the sediment streaked on culture plates. Increased recovery may be enhanced by diluting and plating the specimen serially up to 10⁻³. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.⁽¹⁾ Agar plates should be taped to restrict dehydration. Incubate plates in 5-10% CO₂ at 35°C for up to 10 days.

INTERPRETATION OF RESULTS

Examine inverted plates microscopically at 40X-100X. *Mycoplasma hominis* is recognized by typical "fried-egg" colonies or finely granular colonies with a berry-like appearance that penetrate the agar surface. *M. hominis* colonies range from 20-300µm. *Ureaplasma urealyticum* on A8 Agar is characterized by golden brown, small granular colonies that penetrate the agar surface. *U. urealyticum* colonies range from 15-60µm. Consult listed references for more information regarding cultivation and isolation of mycoplasmas.^(4,5)

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Occasional breakthrough of bacterial growth may occur on medium. Similarities of L-form bacteria and mycoplasma organisms on the agar may cause some confusion because they both exhibit "fried-egg" colonies that penetrate the agar surface. L-form colonies tend to be larger and demonstrate a rougher surface. Many L-form will revert back to the bacterial form if passed to a penicillin-free medium.

Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3} . Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.⁽¹⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Mycoplasma hominis</i> ATCC® 23114	K	72-96hr	35°C	CO ₂ **	Growth observed microscopically at 1-4 days with a "fried-egg" or granular appearance
<i>Ureaplasma urealyticum</i> ATCC® 27618	K	72-96hr	35°C	CO ₂ **	Growth observed microscopically at 1-4 days; golden brown colonies, coarse, granular with irregular edges
<i>Staphylococcus epidermidis</i> ATCC® 12228	B	24hr	35°C	Aerobic	Inhibited
<i>Candida albicans</i> ATCC® 10231	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

** Atmosphere of incubation is enriched with 5-10% CO₂.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

A8 Agar should appear slightly opalescent, and whitish to light amber in color.



Microscopic image of *Mycoplasma hominis*(ATCC[®] 23114) and *Ureaplasma urealyticum* (ATCC[®] 27618) colonies growing on A8 Agar (Cat. no. G02). Incubated in CO₂ for 72 hours at 35°C. All colonies are mycoplasma except for the dark one, which is ureaplasma.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. Clyde, W.A., et al. 1984. *Cumitech 19; Laboratory Diagnosis of Chlamydial and Mycoplasmal Infections*, Coordinating ed., W.L. Drew. American Society for Microbiology, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10000[A]



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

[Ordering Information](#)

Distribution Centers:

California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

Copyright© 1996 by Hardy Diagnostics. All rights reserved.

HDQA 2207F [C]