PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Mycoplasma hominis & *Ureaplasma urealyticum* Culture Procedure

Department of Microbiology

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I. Purpose and Test Principle

Ureaplasma spp. and *Mycoplasma hominis* can be isolated from the lower genital tract in many healthy, sexually active adults, but there is evidence that these organisms are associated with genitourinary tract infection, infertility, premature birth, low birth weight, chorioamnionitis, and congenital pneumonia. Respiratory distress of the newborn has also been associated with ureaplasmas. Genital mycoplasmas have been isolated from throat, cerebrospinal fluid, fetal membranes, bone biopsies, sternal wounds, bronchial washings and tracheal aspirates from neonates, fallopian tubes, products of conception, amniotic fluid, gastric aspirates, and most commonly urogenital specimens.

Mycoplasmas and ureaplasmas cannot be clearly visualized by routine light microscopy. Lack of cell wall precludes visualization by Gram staining. However, colonies grown on specialized growth media can be visualized microscopically. A7 Agar is a solid medium for cultivating and differentiating *M. hominis* and *U. urealyticum* by microscopic observation of colonies. The medium contains growth enrichments and is made selective with the addition of amphotericin B and penicillin. The medium also contains manganous sulfate to detect ammonia produced by the hydrolysis of urea. Urease-positive colonies turn brown due to the formation of particles of manganese dioxide. A7 Agar is inoculated, taped, and incubated at 35 ± 2° C in a CO_2 incubator in a plastic moisture chamber.

10B Arginine Broth is an enriched, urea- and arginine-containing medium for cultivation of both *M. hominis* and *U. urealyticum*. The vial is inoculated and incubated in an ambient air incubator at 35 ± 2° C. The development of a pink color indicates the presence of either *U. urealyticum* due to urea hydrolysis or *M. hominis* due to arginine hydrolysis. The positive vial is then subcultured immediately to A7 agar or refrigerated until it can be subcultured the next day.

II. Specimen Information

A. Specimen Types, Requirements, and Transport
   1. Genital specimens - Urethral swabs from men and vaginal or cervical swabs from women are preferred over urine.
      a. Use only Dacron or polyester swabs with aluminum or plastic shafts.
      b. Place swab in M4 or M6 transport medium.
      c. Transport refrigerated.

2. Fluid specimens (e.g., pleural fluid, NP aspirates or lower respiratory secretions from neonates, CSF, synovial fluid, amniotic fluid, semen, prostatic secretions, and urine).
    a. If the volume of fluid obtained is $\leq$ 2 mL, place fluid in M4 or M6 transport medium, and transport refrigerated.
    b. If the volume is > 2 mL, fluids should be frozen at –70°C in a sterile, leak proof container and shipped on dry ice. Alternatively, specimens may be centrifuged at 600 x *g* and the sediment transferred to M6 transport medium and transported refrigerated.
3. Tissue specimens (e.g., lung, placenta, endometrium, fallopian tube, bone chips, and urinary calculi)
    a. Collect in sterile container with sufficient transport medium to prevent drying.
    b. Transport refrigerated.

B. Specimen Stability
1. Room temp: 8 h
2. Refrigerated: 24 h in M4. If transport will exceed 24 h, freeze sample at –70°C, and transport on dry ice.
3. Frozen: 7 d in M4
4. Frozen (–70°C): 1 month

C. Unacceptable Conditions
1. Transport media other than M4 or M6, including M4 RT
2. Non-patient samples (such as tissue culture cell lines)
3. Dry swabs
4. Wooden-shaft cotton swabs.

III. Reagents & Equipment

- 10B Arginine Broth
- A7 or A8 Agar
- Sterile disposable inoculation (plastic loop) or sterile cotton or dacron swab with plastic-polystyrene shaft.
- Plastic moisture chamber with wet paper towel inserted into lid
- Incubator set at 35 $\pm$ 2°C with 5% CO_2
- Microscope

IV. Procedure

A. Specimen Processing & Media Inoculation
1. Swab received in transport media: Vortex the tube. Inoculate an A7 agar plate by dipping a swab into the specimen and streaking the entire plate 3 times. Rotate the plate 60° after 1st and 2nd streaking. Using a sterile transfer pipette, inoculate a 10B vial with 2-3 drops of transport media.
2. Fluid, including urine, should be centrifuged at 600 x *g* for 15 min. Remove supernatant, and use a drop of the sediment to inoculate the 10B. Use a sterile swab to streak a lawn on the A7 agar.

3. Fluid in transport media should be centrifuged at 600 x *g* for 15 min. Remove supernatant, and use a drop of the sediment to inoculate the 10B. Use a sterile swab to streak a lawn on the A7 agar.
4. Mince tissues with sterile scalpel prior to inoculating media.

B. Incubation

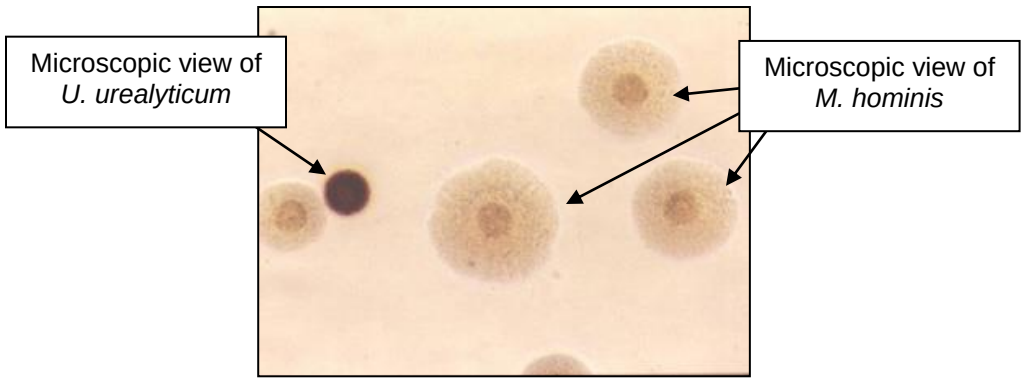
1. Tape A7 agar prior to incubation, and place in a plastic humidity chamber in a 35 ± 2°C CO_2 incubator for up to 7 d. Be sure the chamber lid is NOT snapped shut tightly and a moistened paper towel is inside.
2. Incubate the 10B Arginine vials in a 35 ± 2°C ambient air incubator for up to 7 d and check for color change daily on each shift. **If positive 10B vials are allowed to remain in the incubator longer than 24 h, organism viability is threatened, and cell death usually occurs after an additional 12 h.** Remove any pink vials from the incubator and subculture to A7 agar. Tape the A7 plate, and incubate in a moisture chamber in CO_2 for 5 days.

V. Interpretation and Reporting

A. Interpretation

1. A7 or A8 Agar

The plate should be microscopically examined after 2 to 3 d of incubation and again after 7 d incubation. Examine entire plate for typical colonies using the 10X objective. Brown, granular colonies 15 to 60 μm in diameter that are characteristic of *U. urealyticum* develop in 1 to 3 d. "Fried egg" colonies 200 to 300 μm in diameter that are typical of *M. hominis* develop in 2 to 4 d.



If the primary A7/A8 agar plate becomes overgrown with contaminating organisms and is not readable, discard the plate and continue testing with the 10B Arginine broth as described below. If the 10B Arginine does not produce a color change, the culture is interpreted as negative for both organisms. If the 10B Arginine broth turns pink, and contaminating organisms were observed on the primary A7/A8 agar, subculture the broth as described below. If no *M. hominis* or *U. urealyticum* can be recovered from the broth subculture, consult Rounds.

2. 10B Arginine Broth

Examine broth vials on each shift for color change for up to 7 d. Any vials showing a pink color change should be subcultured as soon as possible to A7/A8 agar.

3. A7/A8 Subculture
   Examine A7 sub plate microscopically for up to 7 d for characteristic colonies.

B. Reporting
   1. Negative Culture
      a. Preliminary report on day 2 or 3:
         No Ureaplasma or Genital Mycoplasma Isolated to Date
      b. Final report on day 7:
         No Ureaplasma or Genital Mycoplasma Isolated
   2. Positive Culture
      a. Preliminary report on day 2 or 3:
         - Ureaplasma urealyticum Isolated.
         - No Mycoplasma hominis Isolated to Date.

         OR
         - Mycoplasma hominis Isolated.
         - No Ureaplasma urealyticum Isolated to Date.

      b. Final report on day 7 (or earlier if both organisms are recovered):
         - Ureaplasma urealyticum Isolated.
         - No Mycoplasma hominis Isolated.

         OR
         - Mycoplasma hominis Isolated.
         - No Ureaplasma urealyticum Isolated.

         OR
         - Mycoplasma hominis Isolated.
         - Ureaplasma urealyticum Isolated.

   3. Indeterminant Results Due to Contamination
      Consult Rounds

VI. Quality Control

A. Control Strains
   1. *Ureaplasma urealyticum* clinical isolate
   2. *Mycoplasma hominis* clinical isolate

B. A7 Agar
   1. Remove A7 agar from the refrigerator, and allow medium to warm to room temperature. Obtain frozen stock of control organisms from freezer, and allow vial to thaw at room temperature.
   2. Both organisms may be inoculated onto the same plate. Dip a sterile swab into the stock cultures, and streak a lawn of inoculum onto the A7 agar.
   3. Tape the plate, and incubate with patient cultures in CO_2 atmosphere inside a moist chamber at 35 $\pm$ 2°C for 3-5 d.
   4. Examine agar for the characteristic colonies described above.
   5. Document QC results in the LIS.

C. 10B Arginine Broth
   1. Remove two 10B Arginine vials, and allow vials to warm to room temperature. Obtain stocks of control organisms from freezer, and allow vials to thaw at room temperature.
   2. Label vials with the names of the respective control organisms.

3. Using a separate sterile transfer pipette, inoculate each vial with a drop of stock. Incubate with patient vials at 35 ± 2°C until a pink color change is visible.
4. Document QC results in LIS

VII. Limitations

A. *U. urealyticum* is susceptible to a rapid, steep death phase in culture, primarily due to urea depletion and elevated pH. Broth cultures showing color changes should be subcultured immediately.
B. 10B Arginine Broth should not be incubated in CO_2 as it contains phenol red indicator.
C. False-positive reactions may occur with the 10B Arginine Broth. They are generally produced by filamentous fungi and *Candida* spp. Subculture to A7 or A8 agar is necessary to confirm the presence of *M. hominis* or *U. urealyticum.*
D. Inhibitory substances in the specimen may prevent the recovery of *M. hominis* and *U. urealyticum*.

VIII. Verification of Test Method

A total of 124 male semen samples were cultured for *Mycoplasma hominis* and *Ureaplasma urealyticum* at SHMC Microbiology, using A7 agar and 10B enrichment broth media incubated for 5 days in CO_2 at 35° C. A total of 20 of the 124 (16%) specimens were positive for *Ureaplasma*, and 2 of the 124 (2%) were positive for *Mycoplasma*.

The current reference laboratory (ARUP) data on genital samples submitted by PAML for the culture of *M. hominis* and *U. urealyticum* were reviewed for a three month period, including from 9/11/02 to 11/06/02 and from 1/14/03 to 2/28/03. A total of 370 samples from males and females were submitted during this 3 month period, with 85 of the 370 (23%) samples positive for *Ureaplasma* and 8 of the 370 (2%) specimens positive for *Mycoplasma*. These data reflect the relative percentage of positive specimens observed with the semen samples cultured at SHMC Microbiology.

In addition, serial dilutions were performed in duplicate using a standardized 10^6 CFU/mL inoculum of *Mycoplasma hominis* (ATCC 23114) in a skim milk diluent. This protocol was performed to compensate for the low percentage of *Mycoplasma* positivity in our patient population. The dilutions were plated onto A7 agar and incubated for 5 days in CO_2 at 35° C. This produced colony counts ranging from 5 to 208 colonies on three different serial dilution plates, yielding final total colony counts ranging from 3.32 to 3.60 x 10^6 CFU/ mL.

In January 2012, an evaluation was performed to compare 10B Arginine broth to the 10B broth that was in use. Specimens submitted for culture were used for inoculating both media. Confirmatory identification was performed by subculture to A7 agar and microscopic examination of colony morphology. A total of 48 specimens were tested. Seventeen (35%) of the specimens were positive for *U.*

urealyticum and/or *M. hominis.* Fifteen (31%) of the specimens were positive for *U. urealyticum.* Four (8%) of the specimens were positive for *M. hominis.* Two (4%) of the specimens were positive for both organisms. The 10B and 10B Arginine performed equally well at detecting *U. urealyticum*. However, 1 of the *M. hominis* isolates was identified only as a result of the positive reaction of the 10B Arginine broth and subsequent subculture and identification on A7 agar. While the prevalence of *M. hominis* remains low, the 10B Arginine broth increased the detection of this organism by 50% in this evaluation. Due to the increased sensitivity and equivalent cost of the 10B Arginine broth, a conversion to 10B Arginine was made.

A parallel study was also performed to compare the performance of A7 and A8 agars. Thirteen previously positive specimens that were frozen at -70 ˚C were thawed and used to inoculate both A7 (Remel) and A8 (Hardy). Two control strains were also used to inoculate both media. A total of 10 *U. urealyticum* and 7 *M. hominis* isolates were recovered on both media. No discrepancies were encountered. However, the growth on A8 agar was larger and faster than that on A7 agar.

IX. References

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G. Package insert: Remel 10B Arginine Broth, IFU 20305-PI, Revised 12/13/2011.

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Revisions & Updates: 01/2012 Switched from 10B to 10B with Arginine. Verified use of either A7 or A8 is acceptable.