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# ISOLATION of GENITAL MYCOPLASMAS

**LD 540 – Hy Mycoplasma Arginine Agar**
**LD 549 – Hy Mycopolasma Urea Agar**
**TT 327 -Hy Mycoplasma Arginine Broth**

## Background and Principle

*U. urealyticum, M hominis, M. fermentans and M. genitalium* are found in human genital tract.
*M. hominis* converts arginine to Ornithine, whereas *U. ureoalyticum* utilizes urea. Thus, two types of media, one containing arginine and the other containing urea are inoculated with the clinical material to be studied.
An aliquot of broth from tubes showing an alkaline shift in pH is subcultured to an agar plate to observe characteristic colony morphology.

## Specimen Collection

***Fluids**: Collect in a sterile container
***Vaginal, Cervical, Urethral swabs**: Collect with a cotton-tipped swab and immediately place the swab into a vial containing about 2-3 ml of a Mycoplasma Transport Medium. Agitate the swab in the medium, express the fluid against the side of the vial, and discard the swab.
***Solid tissues:** Place into a sterile container with sufficient transport medium to prevent drying
* Inoculate specimens as soon after collection as possible, (optimally within 6 hours). Interim storage: 2 - $8^0$ C. For long- term storage: Freeze at $-70^0$C.

## Procedure

**Inoculation in Broths:**
Prepare specimens as described in Specimen Collection and inoculate
TT327 Arginine Broth - (reddish amber) as follows:

**Fluids and Swabs:** 0.2ml of transport medium into 2- 3 ml of each broth
**Tissue:** Place several minced fragments directly into 2 – 3 ml of each broth Medium

1. Cap the tubes, (not tightly) and incubate the cultures aerobically at 35-$37^0$ C. Incubate one inoculated tube in parallel to serve as Reference Color tube.
2. Observe any color change indicating a pH change due to the inoculum BEFORE incubation. Record any change as occurring at time 0.
3. Examine tubes daily for 5 -7 days for a change of pH. An alkaline pH shift will cause the arginine broth from reddish to red. Mycoplasmaloses viability on exposure to the alkaline environment created by the breakdown of arginine.
4. As soon as a rise in pH is noted (color change), subculture the broth (0.1ml),to the appropriate agar plate. Rock the plate to distribute the inoculum evenly on the agar's surface.
5. Incubate the plates inverted, (agar side up), at 35-$37^0$ C in a jar with an atmosphere (5% CO2, 0,5% N2).
6. Observe plates for development of colonies under a 40 –100 magnification with transmitted light. The background of the agar plates becomes red in the zones where Mycoplasma grows:
   *M. hominis:* Fried egg appearance colonies.
   *M. urealyticum:* Small colonies, looking as bits of dust, or large, uneven, textured colonies with black centers.