



Urogenital Mycoplasma Broth Base

M1374

Urogenital Mycoplasma Broth Base (Mycoplasma Urogenital Broth Base) is recommended for selective cultivation of *Mycoplasma hominis* and *Ureaplasma urealyticum*.

Composition**

Ingredients	Gms / Litre
Heart infusion powder	8.000
Casein enzymic hydrolysate	8.000
Yeast extract	4.000
Sodium chloride	3.500
Arginine hydrochloride	5.000
Cysteine hydrochloride	0.100
Phenol red	0.050
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 14.33 grams in 425 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the medium and aseptically add rehydrated contents of 1 vial of Vitamino Growth Supplement (FD025), 1 vial of Urea Solution (FD157), 50 ml Horse serum (RM1239) and 1 vial of Mycoplasma Urogenital Selective Supplement (FD175). Mix well and dispense as desired.

Principle And Interpretation

The two *Mycoplasma* species *Mycoplasma hominis* and *Ureaplasma urealyticum*, have been implicated in urogenital infections in men and women. The organisms are also much smaller than most bacteria measuring 0.2 to 0.3 µm, hence they are able to pass through bacteriological filters. These organisms differ from other bacteria in that they lack a rigid cell wall. Individual cells are bound only by trilaminar unit membrane. Thus cultivation of *Mycoplasma* and *Ureaplasma* requires an enriched medium containing precursors for nucleic acid, protein and lipid biosynthesis. Precursors for nucleic acids and proteins are provided principally by the enriched basal peptone medium and yeast extract, while lipids are provided by the inclusion of serum. In fact, one of the principle criteria used in the taxonomic classification of these organisms is the requirement for the complex lipid cholesterol in the growth medium by certain *Mycoplasma* and *Mycoplasma*-like organisms (7). Urogenital Mycoplasma Broth Base (Mycoplasma Urogenital Broth Base) is based on the formula used by Bebear et al (1, 4), Fiacco et al (2), Bonissol and Daoulas (3), Renaudin et al (5) and Bauriaud et al (6). This medium is used for selective cultivation of urogenital *Mycoplasma*, viz. *M. hominis* and *U. urealyticum* from clinical samples.

The medium contains casein enzymic hydrolysate and heart infusion powder, which provide necessary nutrients for the growth of *Mycoplasma* and *Ureaplasma*. Yeast extract provides preformed nucleic acid precursors, necessary for the growth of fastidious *Mycoplasma*. Many *Mycoplasmas* require serum for their good growth and also presence of antibiotics (present in Mycoplasma Urogenital Selective Supplement, FD175) is necessary to prevent the growth of accompanying microbial flora. Sodium chloride maintains the osmotic balance. Phenol red acts as a pH indicator. *M. hominis* metabolizes arginine to ammonia via ornithine by a three enzyme system, resulting in increase in the pH of the medium which is indicated by a colour change to red. *Ureaplasma* possess the enzyme urease and breakdown urea to ammonia indicated by a colour change to red-orange. Additional tests are required for the differentiation between *M. hominis* and *U. urealyticum*.

U. urealyticum usually causes a colour change in the broth within 24 hours except when the titre is low, the change is observed within 48 hours. *M. hominis* usually causes the colour change within 48 hours. When the titre is high, the colour change occurs within 24 hours. A negative broth should remain clear or may show a faint haze. High turbidity in the broth indicates presence of contaminants.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Reddish pink coloured clear solution in tubes

Reaction

Reaction of 2.86% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH

6.10-6.50

Cultural Response

M1374: Cultural response observed after an incubation at 35-37°C for 48 hours to one week with added Vitamino Growth Supplement (FD025), Urea Solution (FD157), Horse Serum (RM1239) and Mycolasma Urogenital Selective Supplement (FD175).

Organism	Growth	Arginine	Urea
<i>Mycoplasma hominis</i> ATCC 14027	good-luxuriant	positive reaction, red colour	negative reaction, no colour change
<i>Ureaplasma urealyticum</i> ATCC 27618	good-luxuriant	negative reaction, no red colour	positive reaction, red-orange colour

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Bebear C., De Barbeyrac B., Bernet C., Renaudin H., 1989, Ann. Biol. Clin., 47, 415-420.
2. Fiacco V., Miller M. J., Carney E., Martin W. J., 1984, J. Clin. Microbiol., 20, 882-865.
3. Bonissol C., Daoulas-Lebourdelles F., 1979, Sem. Hop. Paris, 13-14, 670-677.
4. Bebear C., Renaudin H., Maugeim J., De Barbeyrac B., Clerc M. T., 1990, Zsntalblatt 20, 77-82.
5. Renaudin H., Quentin C., De Barbeyrac B., Bebear C., 1988, Pathologie Biologie, 36-496-499.
6. Bauriaud R., Seror C., Lareng M. B., Lefevre J. C., 1992, Pathologie Biologie, 40, 479-482.
7. Tully J. G., Taylor-Robinson D., 1986, Pediatr. Infect. Dis. 5: 5292-5295

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