Northeast Laboratory Services

P.O. Box 788
Waterville, Maine 04903-0788
227 China Road
Winslow, Maine 04901

Administrative Offices
Phone: 207-873-7711
Fax: 207-873-7022

Customer Service
Phone: 1-800-244-8378
Fax: 207-873-7022

TECHNICAL PRODUCT INFORMATION

UREAPLASMA MEDIA

INTENDED USE:

Ureaplasma Agar and Broth are recommended for the cultivation and isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis*.

HISTORY/SUMMARY:

Mycoplasma has long been recognized as pathogens. In 1898 Nocard and Roux et al. isolated the infectious agent from the pleural fluid of cattle with contagious bovine pleuropneumonia and they were able to grow it in liquid media. In 1910 Bordet and Borrel et al. succeeded in growing the organisms on solid medium and described the morphological properties of the colonies. When similar organisms were found in other animal species, they were called pleuropneumonia-like organisms or PPLO, and they were later classified as Mycoplasmas. *Mycoplasma mycoides* var. *mycoides*, the etiologic of the cattle disease, is the type species of the Genus Mycoplasma. In 1937, Dienes and Edsall (1) reported the isolation of mycoplasma, probably *Mycoplasma hominis,* from an abscessed Bartholin's gland. This was the first reported recovery of a mycoplasma from a human source. Since then they have been found in the oropharynx, the urogenital tract and the respiratory tract in man and animals.

In 1960 S. Madoff published the definitive description of the isolation, growth and identification of the organisms and their distinction from the bacterial L Forms (2). Mycoplasmas are now known to be widely distributed in nature; there are countless well characterized species and their potential role as pathogens is well documented.

In 1957 Shepard isolated a fastidious group of mycoplasmas (T-strains) from cases of non-gonococcal urethritis (3). These differ from other mycoplasmas in that they produce tiny colonies, they have an optimum pH of 5.5 to 6.0 and they require urea for growth. Now known as Ureaplasma urealyticum, they have also been implicated in cases of infertility, spontaneous abortion (4), stillbirths, congenital pneumonia and perinatal death (5). Mycoplasma hominis, most commonly found in the urogenital tract, have been isolated from cases of postpartum fever, pelvic inflammatory disease, pyelonephritis and in some unusual cases of extragenital infection (6). *M. hominis* as well as *U. urealyticum* can be cultivated on the Ureaplasma Differential Agar. *Mycoplasma genitalium*, more recently described in urogenital infections, require PCR for identification (7).

In 1962 the etiologic agent of primary atypical pneumonia was identified as *Mycoplasma pneumonia* by Chanock, Hayflick and Barile (8). This species is also implicated in other pulmonary and extra-pulmonary complication in adults and children (5).

PRINCIPLES:

The basic medium is a beef-heart broth, supplemented with fresh yeast extract and horse serum. Penicillin is usually added to inhibit other bacterial growth. Genital mycoplasmas grow well under atmospheric conditions. Colonies on agar develop best in an atmosphere of 95% nitrogen and 5% carbon dioxide. *Mycoplasma pneumoniae* have an absolute requirement for the yeast extract supplement for growth. Most other human strains do not have this requirement. Phenol red is incorporated in the media as a pH indicator. Shepard et al. (9) developed the urease color-test medium (U9) for the identification of *Ureaplasma urealyticum* based on its urease enzyme system capable of hydrolyzing urea with the production of ammonia. This medium has been modified by S. Madoff to enhance growth and identification, Phenol red is added to the agar medium to produce a color change from yellow to pink in the presence of Ureaplasma growth. Manganese sulfate has been substituted for the chloride. Urease positive colonies turn brown due to the formation of particles of manganese dioxide.

FORMULA/LITER:

UREAPLASMA DIFFERENTIAL AGAR (U9)	
Casein Digest	17.0 g
Soy Peptone	3.0 g
Dextrose	2.5 g
Dipotassium Phosphate	2.5 g
Sodium Chloride	5.0 g
Manganese Sulfate	0.2 g
Agar	10.0 g
Yeast Extract	2.3 g
L-Cysteine HCL	1.2 mg
Phenol Red	24.0 mg
Horse Serum	239 mL
NEL-X Enrichment	6.0 mL
Urea 10%	1.2 g
Penicillin G	575,000 U
Putrescine	2.2 g
Amphotericin B	1.7 mg
Distilled Water	761 mL

UREAPLASMA BROTH	
Casein Digest	17.0 g
Soy Peptone	3.0 g
Dextrose	2.5 g
Dipotassium Phosphate	2.5 g
Sodium Chloride	5.0 g
Manganese Sulfate	0.2 g
Urea 10%	5.0 mL
NEL-X Enrichment	5.0 mL
Horse Serum	155 mL
Phenol Red	16.0 mg
Ampicillin	50.0 mg
Amphotericin B	4.0 mg
Distilled Water	835 mL

Agar & Broth pH: 6.0 - 6.6 @ 25°C

PRECAUTIONS:

Living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. This product is for In Vitro Diagnostic Use.

STORAGE:

Broth in bottles and agar plates should both be stored at 2-8°C. Broth in tubes should be stored at -10 t0 -20°C. Broth should not be used if color has changed from yellow to pink. Use media prior to expiration date.

SPECIMEN COLLECTION AND PROCEDURES:

It is advisable to inoculate all specimens, swabs as well as exudates, in Ureaplasma Broth and Agar media immediately on arrival in the laboratory to avoid loss of viability. Swabs and exudates may, if necessary, be inoculated into broth and held no longer than four days at 4°C before incubation. Specimens should be streaked over a wide area of the agar plate. Urine specimens are prepared by centrifugation and the sediment inoculated immediately. Agar cultures are incubated anaerobically at 35-37°C. Cultures of Ureaplasma may show a color change in the plate from yellow to pink as early as 24 hours after incubation. Since viability is limited, Ureaplasma broth cultures should be transplanted as soon as the color change begins to show. This may occur as early as 12 to 16 hours after incubation. Although storage conditions have not been established for all strains or species, most Mycoplasmas and Ureaplasma when fully grown can be stored indefinitely at -80°C. Positive broth cultures must be frozen as soon as possible after indicator change has occurred to preserve viability.

IDENTIFICATION:

When seen under the microscope, Ureaplasma colonies produce the manganese reaction turning them dark golden brown or rich deep brown of variable size (depending on crowding). If specimens contain both *Mycoplasma hominis* and *Ureaplasma urealyticum*, differentiation may be enhanced by staining agar block preparations with the Dienes stain (6). Ureaplasma colonies retain the brown color, while *Mycoplasma hominis* and other large colony Mycoplasmas stain azure blue and exhibit the typical "fried egg appearance" due to growth of the organisms into the agar.

RECOMMENDED USER QUALITY CONTROL/PERFORMANCE TESTING:

UREAPLASMA DIFFERENTIAL AGAR [U9] & BROTH

The medium is inoculated by the usual method for mycoplasma. Plates are incubated for 48 hours as specified and examined for growth and manganese reaction.

ORGANISMS:	**RESULTS:**	
TEST ORGANISMS	**GROWTH**	**MANGANESE REACTION**
Ureaplasma urealyticum	+	+
Mycoplasma hominis	+	-
Un-inoculated Control	-	-

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