MYCOPLASMA AGAR BASE

Code: CM0401

A basic medium which, after enrichment with supplements, will support the growth of Mycoplasma species.

Typical Formula*	**gm/litre**
Bacteriological peptone	10.0
`Lab-Lemco' powder	10.0
Sodium chloride	5.0
Mineral supplement	0.5
Agar	10.0
pH 7.8 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

Directions
Add 35.5g to 1 litre of distilled water. Boil to dissolve the agar and distribute in 80ml volumes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add one vial of Mycoplasma Supplement SR0059 reconstituted as directed.

MYCOPLASMA SUPPLEMENT-G

Code: SR0059

POISON - CONTAINS THALLIUM SALT

Vial contents (each vial is sufficient for 80ml of medium)	**per vial**	**per litre**
Horse serum	20.0ml	200.0ml
Yeast extract (25% w/v)	10.0ml	100.0ml
Thallous acetate	25.0mg	250.0mg
Penicillin	20,000IU	200,000IU

MYCOPLASMA SUPPLEMENT-P

Code: SR0060

POISON -- CONTAINS THALLIUM SALT

Vial contents

Horse serum	6.0ml
Yeast extract (25% w/v)	3.0ml
Thallous acetate	0.008g
Glucose	0.3g
Phenol red	0.0012g
Methylene blue chloride	0.0003g
Penicillin	12,000IU
Mycoplasma Broth Base CM403	0.145g

For the preparation of bi-phasic *Mycoplasma pneumonia* medium only.

Directions
Prepare the sterile supplement by aseptically adding 20ml sterile distilled water to the vial and mix gently. Add 1ml of Oxoid Mycoplasma Base without supplements to each of ten small bottles. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set. Aseptically add 2ml of the reconstituted Supplement-P to each bottle containing agar.

Description
Oxoid Mycoplasma Agar Base was formulated as a basal medium to be enriched with any satisfactory supplementary factors used for the growth of mycoplasmas (PPLO).

Edward[1] stressed the importance of the absence of toxic factors to mycoplasmas in the basal medium. Lynn & Morton[2] paid special attention to the inhibitory factors which can be present in batches of agar. Oxoid Mycoplasma Agar Base contains selected constituents shown to be free from such inhibitory or toxic substances. It also contains a special mineral supplement which improves the growth and colony characteristics of mycoplasmas without interfering with the clarity of the medium.

Hayflick[3] suggested inclusion of 10% v/v of a 25% w/v extract of baker's yeast in the medium and Lemcke[4] used Oxoid Yeast Extract LP0021. The majority of mycoplasmas require media enriched with serum; horse serum (20% v/v) is commonly used. Swine or human sera may be substituted for horse serum but the possible presence of antibodies or antibiotics in human serum make media control of great importance (Fallon[6]). The addition of DNA to the medium to encourage the growth of bovine general genital strains and other mycoplasmas was suggested by Edward[7]. 20mg of sodium deoxyribonucleate per ml of medium is quoted by Lemcke[4].

Antibacterial agents are necessary to prevent overgrowth of the slow-growing mycoplasmas by contaminating organisms. Penicillin and thallous acetate are the most common agents used but T-strain *mycoplasma** are sensitive to thallous acetate. Hutchinson[5] and Fallon[6] state that ampicillin at 1mg/ml of medium may be substituted for penicillin and thallous acetate.

Penicillin may be used at concentrations between 50 and 500 units per ml and thallous acetate between 1/2000 and 1/8000 (Lemcke)[4]. It is preferable to omit thallous acetate when searching for T-strain *mycoplasma** (Shepherd & Lanceford[8]).

Two supplements, Mycoplasma Supplement-G SR0059 and Mycoplasma Supplement-P SR0060 have been developed for the improved growth of *mycoplasmas*. Mycoplasma Supplement-G SR0059 is a general supplement prepared to the formulation of Hayflick[3] which, when added to Oxoid Mycoplasma Broth or Agar Base produces a complete selective medium for the propagation of sterol-requiring *Mycoplasma* species of the classical type.

Mycoplasma Supplement-P SR0060 is a liquid supplement based on the formulation recommended by the Mycoplasma Reference Laboratory, CPHLS, Colindale, which is used in conjunction with Mycoplasma Agar Base CM0401 to form a bi-phasic medium for the isolation and preliminary identification of *Mycoplasma pneumoniae*.

Many species of *mycoplasmas* are aerobes or facultative anaerobes but some prefer micro-aerophilic conditions with the addition of carbon dioxide, or strict anaerobiosis.

Pathogenic strains grow best at 35°C while saprophytic strains often grow between 22°C and 30°C, T-strains* have an optimal temperature of 36°C.

Mycoplasma species grow best at pH 7.4-8.0 but T-strains* prefer pH 6.0-6.5.
* **T-strain mycoplasma = *Ureaplasma urealyticum***

Technique
Agar plates
Material for cultivation is inoculated onto agar plates (usually 55mm) prepared with Mycoplasma Agar Base CM0401 + Mycoplasma Supplement-G SR0059. Plates are incubated in moist chambers aerobically, anaerobically and in 10% CO_2-90% N_2 atmosphere.Examine the agar surface after 7 days incubation with a dissecting microscope at 60x magnification, using obliquely transmitted light. The colonies are characteristic with the centre of the colony embedded beneath the surface, giving a `fried-egg' appearance.

Purification of the organism by further cloning sub-cultures is essential before identification. This may be carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium. Growth inhibition tests using specific antisera may then be carried out (Clyde[9]).

BI-phasic Medium
Bi-phasic media preparedwith 1ml quantities of solid Mycoplasma Agar Base overlaid with 2ml of reconstituted Mycoplasma Supplement-P SR0060. Bi-phasic medium bottles should be inoculated with a swab or a fleck of sputum and incubated at 35°C for up to three months. Any bottles showing gross turbidity due to growth of bacteria or fungi should be discarded.

Growth of *Mycoplasma pneumoniae* results in the reduction of methylene blue followed by production of acid due to the fermentation of glucose, resulting in a colour change of the phenol red indicator to yellow. Bottles showing such a colour change should be sub-cultured onto agar for further examination.Mycoplasma Agar Base supplemented with Mycoplasma Supplement-G SR0059 is suitable for this purpose.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Straw coloured gel

Quality Control

Positive control:	**Expected result at 35°C**
Mycoplasma pneumoniae ATCC® 15531	Microscopic examination- 'fried-egg' colonies
Negative control:	
Escherichia coli ATCC® 25922 *	Inhibited

*** This organism is available as a Culti-Loop®**

Precautions
Thallous acetate is toxic, observe the precautions in the HAZARDS section.
Do not use thallous acetate media to isolate *Ureaplasma urealyticum*.
Mycoplasmas may be suspected if (1) typical morphology (2) no growth in media without serum (3) colonies are embedded below the agar surface.

References

1. Edward D. G. ff. (1971) *J. Gen. Microbiol*. 69. 205-210.
2. Lynn R. J. and Morton H. E. (1965) *Appl. Microbiol*. 4. 339-341.
3. Hayflick L. (1965) *Texas Rep. Biol. & Med. 23. suppl*. 1. 285-303.
4. Lemcke Ruth M. (1965) *`Media for the Mycoplasmataceae', Lab. Pract*. 14. 712.
5. Hutchinson D. (1969) *J. Med. Lab. Technol*. 26. 111-116.
6. Fallon R. J. (1969) *S. A. B. Technical series 3. Academic Press*. 41-50.
7. Edward D. G. ff. (1954) *J. Gen. Microbiol*. 10. 27-64.
8. Shepard M. C. and Lanceford C. D. (1970) *Appl. Microbiol*. 2. 539-543.
9. Clyde W. A. (1964) *J. Immun*. 92. 958-962.

New York City Medium for Mycoplasmacaceae.
NYC Medium, primarily designed for the isolation of pathogenic *Neisseria*, also readily supports the growth of *Mycoplasma* and *Ureaplasma* species.

The transparent, highly selective medium permits direct microscopic observation and presumptive recognition of *mycoplasmas*. As a single medium it can, therefore, be doubly useful in the diagnosis of gonorrhoea and in the recognition of active or asymptomatic mycoplasma infections.
Urine specimens can be cultivated on NYC Medium to detect the presence of *Ureaplasma* species (T-strain *mycoplasma*).
See GC Selective Media Section in the Manual.

References

1. Faur Y. C., Weisburd M. H., Wilson M. E. and May P. S. (1974) *Appl. Microbiol*. 27. 1041-1045.
2. Hipp S. S., Rockwood L. D., Gaafar H. A. and Han Y. (1981) *J. Clin. Microbiol*. 13. 135-138.