PRODUCT SPECIFICATION SHEET

Mycoplasma Broth Base w/ CV (PPLO Broth Base w/ CV) (DM175)

Intended Use

Mycoplasma Broth Base w/ CV (PPLO Broth Base w/ CV) (DM175) with addition of enrichment it is used for isolating *Mycoplasma* species (PPLO) from clinical specimens and mixed cultures.

Product Summary and Explanation

Among the members of class *Mollicutes*, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow. The organism was designated "pleuropneumonia-like organism," or PPLO.[1] Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").[1] *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract. PPLO (Mycoplasma) Media were described by Morton, Smith and Leberman.[2] It was used in a study of the growth requirements of *Mycoplasma*,[3] along with the identification and cultivation of this organism.[4-6] Pivotal information regarding *Mycoplasma* has been documented by Sabin.[7] Hayflick et al have reported the information regarding the cultivation of *Mycoplasma*.[8]

Principles of the Procedure

Mycoplasma Agar Base contains beef heart infusion, peptic digest of animal tissue and peptone which provides nitrogen, vitamins, amino acids and carbon required for growth. Sodium chloride maintains the osmotic balance of these formulations. Crystal violet and potassium tellurite inhibits many gram-negative and gram-positive bacteria. Many *Mycoplasma* require serum for their good growth and also presence of antibiotic is necessary to prevent the growth of contaminating organisms. Mostly the *Mycoplasma* species are aerobic or facultatively anaerobic but some are microaerophilic. Few are anaerobic saprophytic *Mycoplasma* which grow best at 22-35°C while pathogenic strains grow at 35°C. *Mycoplasma* when grow in the agar medium show typical morphology and form colonies below the agar surface and do no grow without serum.

Formula / Liter

Ingredients	Gms / Liter
Beef heart, infusion from	250.00
Peptic digest of animal tissue	10.00
Sodium chloride	5.00
Crystal violet	0.01
Final pH: 7.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. For the cultivation of *Mycoplasma* the medium ingredients and all the supplements should be free of any toxic substances even in small amounts.

Directions

1. Suspend 21 grams of the medium in 700 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45°C and aseptically add 300 ml Horse serum (MS084) or 10 vials of Mycoplasma Enrichment Supplement (MS190). Mix well before dispensing.
5. Add 2.85 ml of Potassium Tellurite Supplement 1% (MS024) along with MS084 or MS190. 25% Ascitic fluid can be used instead of Horse serum.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured may have purple tinge, clear solution in tubes
Reaction of 2.1 % solution	pH 7.8 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed in presence of 10% Carbon dioxide with added ,1% Horse serum (MS084) or 10 vials of Mycoplasma Enrichment Supplement (MS190), after an incubation at 22-35°C for 48 hours.

Sr. No.	Organisms	Results to be achieved
		Growth
1.	*Mycoplasma bovis ATCC 25523*	good-luxuriant
2.	*Mycoplasma gallinarium ATCC 19708*	good-luxuriant
3.	*Mycoplasma pneumoniae ATCC 15531*	good-luxuriant
4.	*Streptococcus pneumoniae ATCC 6303*	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the surface of plates containing the complete medium by adding drops of liquid inoculum or by a swab-inoculation technique.
2. Incubate plates at 35 ± 2°C in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M. buccale*, *M. faucium*, *M. orale* or *M. salivarium* is suspected. Examine after incubation of 48 hours but they should not be discarded as negative until after incubation for 3 weeks.
3. Refer to appropriate references for standard test procedures.

Results

1. PPLO colonies are round with a dense center and a less dense periphery, resembling a "fried egg" on PPLO Agar. Vacuoles, large bodies characteristic of *Mycoplasma* species are seen in the periphery.
2. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.
3. Refer to appropriate references and standard test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : Mycoplasma Broth Base w/ CV (PPLO Broth Base w/ CV)
Product Code : DM175
Available Pack sizes : 500gm

References

1. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
2. Morton, Smith and Leberman. 1951. Am. J. Syphilis Gonorrh. *35*:361.
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4. Chanock, James, Fox, Turner, Mufso and Hayflick. 1962. Soc. Exp. Biol. Med. *110*:884.
5. Craven, Wenzel, Calhoun, Hendley, Hamory and Gwaltney. 1976. J. Clin. Microbiol. *4*:225.
6. Gregory and Cundy. 1970. Appl. Microbiol. *19*:268.
7. Sabin, 1941, Bacteriol. Rev., 5:1, 331.
8. Hayflick and Chanock, 1965, Bacteriol, Rev., 29:185.

Further Information

For further information please contact your local MICROMASTER Representative.

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