PPLO AGAR w/ and w/o METHYLENE BLUE

INTENDED USE

Remel PPLO Agar w/ and w/o Methylene Blue are solid media recommended for use in qualitative procedures for the cultivation and selective isolation of *Mycoplasma* species.

SUMMARY AND EXPLANATION

The pleuropneumonia-like organisms (PPLO) are a group of pleomorphic, filterable, fastidious microorganisms which also lack a rigid cell wall. They were first studied by Nocard et al. in 1898 and determined as the causative agent of bovine pleuropneumonia. Soon, PPLO was documented from other sources and in 1956 Edward and Freundt proposed the recent system of classification and nomenclature for the PPLO, now referred to as *Mycoplasmataceae*.[1,2] Morton, Smith, and Leberman formulated PPLO agar base for the isolation and cultivation of *Mycoplasma* species.[3] Hayflick modified this formulation by the addition of yeast extract and unheated horse serum.[4] In 1965, Kraybill demonstrated *M. pneumoniae* is resistant to methylene blue in agar medium, while other species of *Mycoplasma* are inhibited.[5,6]

PRINCIPLE

This medium contains beef heart infusion and peptone to supply nutrients required for the growth of mycoplasmas. Sodium chloride maintains osmotic equilibrium. Yeast extract supplies a variety of B-complex vitamins and enhances growth. Normal horse serum provides a protein source. Penicillin and thallium acetate are added as inhibitors of bacteria. Yeasts and molds are inhibited by the amphotericin B contained in the medium. Methylene blue is added to inhibit species of *Mycoplasma* other than *M. pneumoniae*.

REAGENTS (CLASSICAL FORMULA)*

Beef Heart Infusion	50.0 g	Horse Serum	200.0 ml
Peptone	10.0 g	Yeast Extract 25%	100.0 ml
Sodium Chloride	5.0 g	Penicillin	1,000,000 U
Thallium Acetate	0.25 g	Agar	14.0 g
Amphotericin B	2.5 mg	Demineralized Water	700.0 ml

The following ingredient is added per liter of PPLO Agar w/ Methylene Blue:
Methylene Blue Solution 1% ..... 0.2 ml

pH 7.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Prepare a 1:10 dilution of the specimen in a suitable broth medium, such as PPLO Broth (REF R20360).[7]
2. Using a sterile pipette, transfer an aliquot (0.2 ml) of the broth to PPLO Agar w/ or w/o Methylene Blue.
3. Streak the plate for isolation and seal closed to restrict dehydration.
4. Incubate the plate at 35-37°C in 5% CO_2 for up to 4 weeks.[7]
5. Examine PPLO Agar microscopically for typical colonial morphology (10-500 µm in diameter), at 1-3 day intervals for *M. hominis,* and every 3-5 days for *M. pneumoniae* and other slower-growing species. *M. hominis* colonies exhibit a typical "fried-egg" appearance consisting of an opaque, granular central zone embedded in the agar and a flat, translucent peripheral zone. Other species, such as *M. pneumoniae*, produce smaller spherical colonies, which may or may not demonstrate the "fried-egg" appearance.[7]
6. Examine PPLO Agar w/ Methylene Blue for typical *M. pneumoniae* colonies as described for PPLO Agar; other species of *Mycoplasma* pathogenic in humans, are inhibited by methylene blue.[5,6]

Note: Serial dilutions to 10^{-3} of specimen will optimize recovery; dilution is recommended to overcome potential inhibitory substances that may be present in the medium or in the specimen.[6]

QUALITY CONTROL

All lot numbers of PPLO Agar w/ and w/o Methylene Blue have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Mycoplasma pneumoniae ATCC® 15531	CO_2, 3-5 days @ 35-37°C	Growth recovered on subculture
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 35-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 35-37°C	Inhibition (partial to complete)
Candida albicans ATCC® 10231	Ambient, 18-24 h @ 35-37°C	Inhibition (partial to complete)

LIMITATIONS

1. Occasional breakthrough of bacterial growth may occur on this medium.
2. Thallium acetate has been demonstrated to inhibit ureaplasmas and *Mycoplasma genitalium.*[8]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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