PPLO BROTH
SELECTIVE AND NONSELECTIVE

INTENDED USE
Remel PPLO Broth Selective and Nonselective are liquid media recommended for use in qualitative procedures for the cultivation 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.[1] They were first studied by Nocard et al. in 1898 and determined to be the causative agent of bovine pleuropneumonia. Soon, PPLO were documented from other sources and in 1956 Edward and Freundt proposed the recent system of classification and nomenclature for PPLO which now contains a single family, *Mycoplasmataceae.* Though most are considered normal commensal flora a few have become well established pathogens, causing potentially serious complications of disease.[2] This has lead to an increase in demand for laboratory identification of these organisms. The extremely small amount of genetic material possessed by PPLO makes them demanding in their nutritional requirements for cultivation. 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]

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. Horse serum provides a protein source. Penicillin and thallium acetate may be added as inhibitors of bacteria. Yeast and molds are inhibited by adding amphotericin B to the medium.

REAGENTS (CLASSICAL FORMULAE)*
Beef Heart Infusion	50.0 g	Sodium Chloride	5.0 g
Yeast Extract	25.0 g	Horse Serum	200.0 ml
Peptone	10.0 g	Demineralized Water	800.0 ml

pH 7.8 +/- 0.2 @ 25°C

The following ingredients are added per liter of PPLO Broth, Selective:

Penicillin	1,000,000 U	Thallium Acetate	0.25 g
		Amphotericin B	2.5 mg

*Adjusted as required to meet performance standards.

PROCEDURE
Specimen Preparation:
1. Body Fluids
   a. Concentrate fluids by centrifugation at 1500 rpm for 15 minutes.
   b. Use concentrate to inoculate PPLO Broth, Selective or Nonselective.
   c. Transfer 0.5-1.0 ml of concentrated sample into broth using a sterile pipette.
   d. If centrifugation is not possible, inoculate fluid into broth in a 1:10 ratio.
   e. Sputum may be inoculated directly into the broth.
2. Tissue
   a. Mince tissue into fragments using a sterile scalpel. Avoid grinding, as it tends to pulverize tissue, releasing growth inhibitors.
   b. Place minced tissue directly into the broth.
3. Blood
   a. Collect blood free of anticoagulants.
   b. Immediately inoculate into broth in a 1:5 to 1:10 ratio (preferably 5-10 ml for adults).
4. Swab Specimens
   a. Place swab in broth and swirl.
   b. Express excess liquid by pressing swab against the inside of the tube.
   c. Discard the swab.

Inoculation:
1. For optimal recovery, serially dilute the specimen in PPLO Broth to at least 10^{-3} (i.e., 0.2 ml of sample in 1.8 ml of broth).
2. Using a sterile pipette, transfer an aliquot (0.2 ml) of each, inoculated broth and serial dilution(s), to appropriate plated media, such as SP4 Glucose Agar (REF 20276) or PPLO Agar (REF 20260).
3. If possible, freeze remainder of original sample at -70°C for future confirmation.

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.[2,6]

Incubation:
1. Seal plate(s) to prevent dehydration and incubate in 5% CO_2 at 35-37°C for up to 4 weeks.[6]
2. Examine microscopically for typical colonial morphology (10-500 µm in diameter), at 1-3 day intervals for *Mycoplasma hominis*, and every 3-5 days for *Mycoplasma 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.[6]

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QUALITY CONTROL

All lot numbers of PPLO Broth Selective and Nonselective 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
PPLO Broth:		
Mycoplasma pneumoniae ATCC® 15531	CO_2, up to 5 days @ 35-37°C	Growth recovered on subculture
PPLO Broth, Selective:		
Mycoplasma pneumoniae ATCC® 15531	CO_2, up to 5 days @ 35-37°C	Growth recovered on subculture
Candida albicans ATCC® 10231	Aerobic, 24 h @ 35-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Aerobic, 24 h @ 35-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, 24 h @ 35-37°C	Inhibition (partial to complete)

LIMITATIONS

1. Thallium acetate has been demonstrated to inhibit ureaplasmas and *Mycoplasma genitalium*.[2,5]

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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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