
SP4 GLUCOSE BROTH

INTENDED USE

Remel SP4 Glucose Broth is a liquid medium recommended for use in qualitative procedures for the cultivation of *Mycoplasma* species, including *Mycoplasma pneumoniae*.

SUMMARY AND EXPLANATION

Nocard first described *Mycoplasma* in 1898 as the causative agent of bovine pleuropneumonia and designated the name "pleuropneumonia-like organisms (PPLO)". *Mycoplasma* spp. are much smaller than most bacteria and are distinguished by the lack of a cell wall. Though most are considered commensal microbial flora, a few have become well-established pathogens with the potential for causing infections with serious complications.^{1,2} Because these organisms possess an extremely small amount of genetic material, their nutritional requirements for cultivation are very high. PPLO medium, developed by Hayflick in 1965, supports the growth of all strains of *Mycoplasma*.³ In 1977, Tully et al. developed SP4 Glucose Broth by adding fetal bovine serum to PPLO medium.⁴

PRINCIPLE

SP4 Glucose Broth contains beef heart infusion, peptone, CMRL 1066 medium, and fetal bovine serum which supply the nutrients required for the growth of *Mycoplasma* spp. Yeast extract supplies a variety of B-complex vitamins and enhances growth. Fetal bovine serum provides cholesterol and protein. Glucose is metabolized by some *Mycoplasma* spp., including *M. pneumoniae*, causing the phenol red indicator to change from red to yellow as a result of an acid shift. Penicillin and thallium acetate are selective agents which inhibit many bacteria other than *Mycoplasma* spp.

REAGENTS (CLASSICAL FORMULA)*

Tryptone	10.0 g	CMRL 1066	0.49 g
Yeast Extract	8.75 g	Thallium Acetate	0.25 g
Peptone	5.3 g	Phenol Red	20.0 mg
Glucose	5.0 g	Penicillin	1,000,000 U
PPLO Broth Base	3.5 g	Fetal Bovine Serum	170.0 ml
Yeastolate	2.0 g	Demineralized Water	615.0 ml

pH 7.5 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

Specimen Preparation:

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

Inoculation:

- For optimal recovery, serially dilute the specimen in SP4 Glucose Broth to at least 10⁻³ (for example, 0.2 ml of sample in 1.8 ml of broth).
- Using a sterile pipette, transfer an aliquot from the dilution (0.2 ml) onto plated media, such as SP4 Glucose Agar (R20276) or PPLO Agar (R20260).
- If possible, freeze remainder of original sample at -70°C for future confirmation.

NOTE: Serial dilutions to 10⁻³ of any specimen will optimize recovery and is recommended to overcome potential inhibitory substances that may be present in the medium or in the specimen.^{1,5}

Incubation:

- Broth Media
 - Incubate all broth dilution tubes in ambient air at 35-37°C for up to 4 weeks; observe daily.
 - After any color change (from red to yellow), subculture an aliquot (0.2 ml) onto plated media such as SP4 Glucose Agar or PPLO Agar.
 - If possible, freeze positive broth cultures at -70°C immediately after subculture for future reference.
- Plated Media
 - Seal plate(s) to prevent dehydration and incubate in 5% CO₂ at 35-37°C for up to 4 weeks.⁵
 - Examine 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.⁵

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QUALITY CONTROL

All lot numbers of SP4 Glucose Broth 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

Mycoplasma pneumoniae ATCC® 15531
Escherichia coli ATCC® 25922
Staphylococcus aureus ATCC® 25923

INCUBATION

CO₂, up to 5 days, 33-37°C
Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C

RESULTS

Yellow color change, good recovery on subculture
Inhibition (partial to complete)
Inhibition (partial to complete)

LIMITATIONS

1. Occasional breakthrough of bacterial growth may occur.
2. Thallium acetate has been demonstrated to inhibit *Ureaplasma* spp. and *Mycoplasma genitalium*.⁵
3. Subcultures from broth to agar must be done before color change is complete.⁶

BIBLIOGRAPHY

1. Cassell, G.H., K.B. Waites, and D.T. Crouse. 1995. *Infectious Diseases of the Fetus & Newborn*. 4th ed. W.B. Saunders Co., Philadelphia, PA.
2. Howard, B.J., J.F. Keiser, T.F. Smith, A.S. Weissfeld, and R.C. Tilton. 1994. *Clinical and Pathogenic Microbiology*. 2nd ed. Mosby, St. Louis, MO.
3. Hayflick, L. 1965. *Texas Rep. Biol. Med.* 23:285.
4. Tully J.G., D.L. Rose, R.F. Whitcomb, and R.P. Wenzel. 1979. *J. Infect. Dis.* 139:478-482.
5. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock. 2011. *Manual of Clinical Microbiology*. 10th ed. ASM Press, Washington, D.C.
6. MacFaddin, J.F. 1985. *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*. Vol. 1. Williams and Wilkins, Baltimore, MD.

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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