SP4 GLUCOSE AGAR

INTENDED USE
Remel SP4 Glucose Agar is a solid 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 Agar by adding fetal bovine serum to PPLO medium.[4]

PRINCIPLE
SP4 Glucose Agar 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	Agar	8.0	g
			Demineralized Water	615.0	ml

pH 7.5 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prepare a 1:10 dilution of the specimen in a suitable transport medium, such as SP4 Glucose Broth (R20376), following recommended guidelines.[5]
2. Using a sterile pipette, transfer an aliquot (0.2 ml) from the broth to the SP4 Glucose Agar.
3. Streak the plate for isolation and seal closed to restrict dehydration.
4. Incubate SP4 Glucose Agar at 35-37°C in 5% CO_2 for up to 4 weeks.[5]
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]

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]

QUALITY CONTROL
All lot numbers of SP4 Glucose Agar 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, up to 5 days, 33-37°C	Good growth
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

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

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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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