SP4 MEDIA

Cat. no. G21	SP4 Agar with Glucose, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. G32	SP4 Agar with Arginine, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. R85	SP4 Broth with Arginine, 13x100mm Tube, 2ml	20 tubes/box
Cat. no. R86	SP4 Broth with Glucose, 13x100mm Tube, 2ml	20 tubes/box
Cat. no. R87	SP4 Broth with Urea, 13x100mm Tube, 2ml	20 tubes/box
Cat. no. U86*	SP4 Broth with Glucose, 125ml Polypropylene Bottle, 90ml	1 each
Cat. no. U513*	SP4 Broth with Urea, 500ml Polycarbonate Bottle, 500ml	1 each

INTENDED USE

Hardy Diagnostics SP4 Media are recommended for the isolation, differentiation and maintenance of mycoplasma, including *M. hominis, M. pneumoniae,* and *Ureaplasma urealyticum.*

*Bottle format not useful for clinical procedures.

SUMMARY

SP4 Media are highly nutritious due to the addition of beef heart infusion, peptone supplemented with yeast extract, CMRL 1066 Medium, and fetal bovine serum. Yeast extract provides diphosphopyridine nucleotides and serum provides cholesterol and an additional source of protein. Specific substrates, such as glucose, arginine, or urea, may be added and used in conjunction with pH to differentiate and select for certain mycoplasma. Amphotericin B, polymyxin B, and penicillin are added to inhibit faster growing contaminants. Phenol Red is added to broth media as a pH indicator.

FORMULA

Ingredients per 690ml of deionized water:*

SP4 Broth, Base:**	
Pancreatic Digest of Casein	10.0gm
Pancreatic Digest of Gelatin	5.0gm
PPLO Broth without CV	3.5gm
Polymyxin B	50.0mg
Amphotericin B	5.0mg

Fetal Bovine Serum	170.0ml
CMRL 1066 Medium (10X)	50.0ml
Yeast Extract	35.0ml
Yeastolate 10%	20.0ml
Penicillin	1,000,000U

Note: In addition to the above ingredients;

SP4 Broth with Glucose contains:**	
Glucose	5.0gm/L

SP4 Broth with Arginine contains:**	
Arginine	5.0gm/L

SP4 Broth with Urea contains:**	
Urea	1.0gm/L

SP4 Agar Media contains:	
Agar	9.0gm/L

****All broth media contain:**	
Phenol Red	18.0mg/L

Final pH at 25°C:

SP4 Broth with Arginine
7.0 +/- 0.2

SP4 Agar with Glucose
SP4 Broth with Glucose
7.4 +/- 0.2

SP4 Broth with Urea
6.0 +/- 0.2

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The

product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

Product nos. G21, G32, R85, R86, and R87:

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Product nos. U86 and U513:

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimen/swab should be placed in a tightly sealed transport container with sufficient transport medium to prevent drying. Samples should be taken directly to the laboratory and processed as soon as possible. If there is to be a delay in culturing, specimens should be refrigerated. For long-term storage, or if cultures cannot be set up within 24 hours of collection, freeze specimens at -70°C in a cryopreservative such as Brucella with Glycerol (Cat. no. D04). Do not freeze at temperatures warmer than -70°C Consult listed references for more information regarding cultivation and isolation of mycoplasmas.

SP4 Agar Media

1. If using a liquid inoculum, add 0.1ml of liquid to the agar surface and distribute evenly by rocking the plate back and forth or spreading the inoculum with a sterile bent glass rod. If culturing directly from a swab, roll the swab over the agar surface and streak for isolation. Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[1]

2. Apply tape or similar sealant over agar plates to restrict dehydration.

3. Incubate plates in 5-10% CO_2 at 35°C for up to 30 days.

4. Invert plates and examine microscopically at 100x magnification.

5. Observe for typical tiny "fried-egg" colonies or finely granular colonies with a berry-like appearance that penetrate the agar surface. Colonies range from 20-300µm in diameter.

SP4 Broth Media

1. Inoculate broth with 0.1ml of transport media containing swab. Alternatively, broth may be inoculated at a 1:10 ratio with blood or CSF. Tissue specimens may be inoculated by placing several minced fragments directly into the broth. Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[1]

2. Tighten cap and incubate inoculated broth aerobically at 35°C for up to 30 days.

3. Examine tubes daily for a change in the pH, detectable by a color change in the medium.

4. As soon as a pH shift is noted, subculture the broth to an appropriate agar medium. Consult listed references for more information regarding cultivation and isolation of mycoplasma.

INTERPRETATION OF RESULTS

SP4 Agar Media

Mycoplasma appear as tiny "fried-egg" colonies or as finely granular colonies with a berry-like appearance that penetrate the agar surface. Colonies range from 20-300µm in diameter.

SP4 Broth Media

Growth of glucose fermenting mycoplasma will cause SP4 with Glucose to turn from its original red color to yellow.

Ureaplasmal growth in SP4 with Urea will turn the medium from yellow-orange to red.

Growth of arginine using mycoplasma in SP4 with Arginine results in the medium changing from orange to red.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Occasional breakthrough of bacterial growth may occur on these media. Similarities of L-form bacteria and mycoplasma organisms on the agar medium may cause some confusion because they both exhibit "fried egg" colonies that penetrate the agar surface. L-form colonies tend to be larger and demonstrate a rougher surface. Many L-forms will revert back to the bacterial form if passed to a penicillin-free media.

Increased recovery may be enhanced by diluting and plating the specimen serially up to 10^{-3}. Diluting the specimen minimizes the effect of bacterial inhibitors on the growing mycoplasma.[1]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
The following organisms are inoculated to all media listed below:					
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 25923	B	24hr	35°C	Aerobic	Inhibited
Candida albicans ATCC® 10231	B	24hr	35°C	Aerobic	Inhibited
SP4 Agar with Glucose:					
Mycoplasma pneumoniae ATCC® 15531	K	3-20 days	35°C	CO_2**	Growth; appears as tiny "fried-egg" colonies or finely granular berry-like colonies that penetrate the agar surface, media changes from its original salmon to yellow
Mycoplasma arginini ATCC® 23838	K	3-20 days	35°C	CO_2**	Growth; appears as tiny "fried-egg" colonies or finely granular berry-like colonies that penetrate the agar surface, media will not have a color change
SP4 Agar with Arginine:					
Mycoplasma hominis ATCC® 23114	K	1-4 days	35°C	CO_2**	Growth; appears as tiny "fried-egg" colonies or finely granular berry-like colonies that penetrate the agar surface, media changes from its original red to pink

SP4 Broth with Arginine:					
Mycoplasma hominis ATCC® 23114	K	1-4 days	35°C	Aerobic	Growth; color change in medium from its original orange to red, subculture to appropriate agar medium
SP4 Broth with Urea:					
Ureaplasma urealyticum ATCC® 27618	K	1-4 days	35°C	Aerobic	Growth; color change in medium from its original yellow-orange to red, subculture to appropriate agar medium
SP4 Broth with Glucose:					
Mycoplasma pneumoniae ATCC® 29085	K	3-20 days	35°C	Aerobic	Growth; color change in medium from its original red to yellow, subculture to appropriate agar medium
Mycoplasma arginini ATCC® 23838	K	3-20 days	35°C	Aerobic	Growth, slight turbidity; no color change; subculture to appropriate agar medium
*Mycoplasma hyorhinis*** ATCC® 17981	K	3-20 days	35°C	Aerobic	Growth; color change in medium from its original red to yellow, subculture to appropriate agar medium
*Mycoplasma orale**** ATCC® 23714	K	3-20 days	35°C	Aerobic	Growth, slight turbidity; no color change; subculture to appropriate agar medium

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% CO_2

*** Not tested on product no. R86.

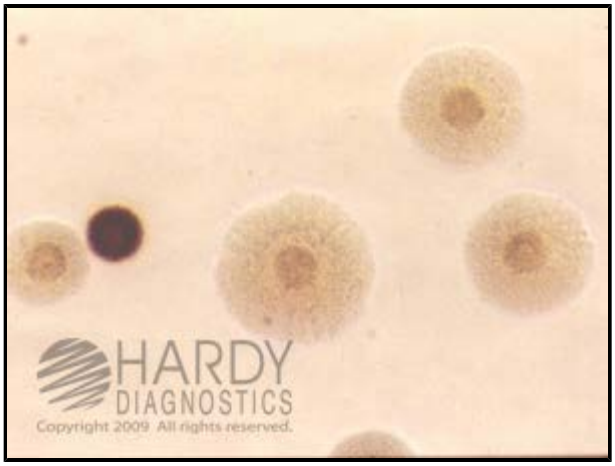
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific

information.

PHYSICAL APPEARANCE

- SP4 Agar with Arginine should appear clear, slightly opalescent, and salmon in color.
- SP4 Agar with Glucose should appear clear, slightly opalescent, and salmon in color.
- SP4 Broth with Arginine should appear clear, slightly opalescent, and reddish-orange in color.
- SP4 Broth with Glucose should appear clear, slightly opalescent, and pink to red in color.
- SP4 Broth with Urea should appear clear, and yellow-orange in color.



Microscopic image of *Mycoplasma hominis* (ATCC® 23114) and *Ureaplasma urealyticum* (ATCC® 27618) colonies growing on SP4 Agar with Glucose (Cat. no. G21). Incubated in CO_2 for 72 hours at 35°C. All colonies are mycoplasma except the dark one, which is ureaplasma.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory,* Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

4. *Cumitech 19; Laboratory Diagnosis of Chlamydial and Mycoplasmal Infections.* American Society for Microbiology, Washington D.C., August, 1984.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media,* M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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HDQA 2207F [C]