Limitations of the Procedure

(See the product insert packaged with PKU Test components for a complete list.)

1. Positive results obtained by this methodology require confirmation by quantitative blood assay.
2. PKU Test Agar must not be overheated. Bring to a boil and mix gently during heating. DO NOT AUTOCLAVE.
3. Do not add spores if the temperature of the medium is above 55°C. Distribute the spores uniformly in the medium without creating bubbles.
4. Place the Petri dish on a horizontal surface while pouring the medium to ensure an even depth of agar and a uniform distribution of spores throughout the plate.
5. Caution and proper controls should be used when utilizing components purchased individually or from other suppliers.

References

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Availability

Difco™ PKU Test Agar
Cat. No. 298010 Dehydrated – 500 g

Difco™ PKU Test Agar without Thienylalanine
Cat. No. 247410 Dehydrated – 500 g

BBL™ ß-2-thienylalanine, 171 mg% aqueous solution
Cat. No. 212105 Tube – 10 × 10 mL*

PKU Subtilis Spore Suspension
Cat. No. 212901 Vial – 100 × 1 mL*

BBL™ PKU Control Disc Strips: Concentrations 2, 4, 4, 4, 4, 4, 6, 8, 10, 12, 20 mg%
Cat. No. 231110 Strips – Pkg. of 10*

BBL™ PKU Test Specimen Cards
Cat. No. 231111 Cards – Pkg. of 1000

BBL™ PKU Tray, complete with template for placing disks
Cat. No. 260435 One

**Store at 2-8°C.*

PPLO Media (Mycoplasma Media)
PPLO Agar (Mycoplasma Agar Base)
PPLO Broth (Mycoplasma Broth Base)
Mycoplasma Broth Base (Frey) • Mycoplasma Supplement • Mycoplasma Enrichment w/o Penicillin

Intended Use

PPLO (Mycoplasma) agars and broths, when supplemented with nutritive enrichments, are used for isolating and cultivating *Mycoplasma*. Mycoplasma Broth Base (Frey) is used for the cultivation of avian mycoplasmas.

Summary and Explanation

Members of the class *Mollicutes*, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow.[1] The organism was designated "pleuropneumonia-like organism," or PPLO.[1] Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").[1] *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract.[1]

PPLO (Mycoplasma) Agar was described by Morton, Smith and Leberman.[2] It was used in a study of the growth requirements of *Mycoplasma*,[3] along with the identification and cultivation of this organism.[4-6]

PPLO (Mycoplasma) Broth (without crystal violet) is prepared according to the formula described by Morton and Lecci.[3] Crystal violet is omitted from this formula due to its inhibitory action on some *Mycoplasma*. It has been used for the cultivation of *Mycoplasma* for research studies.[7,8]

Mycoplasma Broth Base (Frey), a modification of other broth media, was developed specifically for the cultivation of avian strains of *Mycoplasma*.[9]

Mycoplasma Supplement and Mycoplasma Enrichment w/o Penicillin are sterile desiccated enrichments for use in PPLO media as described by Hayflick.[10] The supplements are prepared according to the formulations of Chanock, Hayflick and Barile[11] and Hayflick.[12]

Principles of the Procedure

Meat digests, peptones, beef extract and yeast extract provide the nitrogen, vitamins, amino acids and carbon in these media. Sodium chloride maintains the osmotic balance of these formulations. Agar, the solidifying agent, is used in PPLO (Mycoplasma) Agar at a concentration slightly reduced from usual to ensure formation of the largest possible colonies because the organisms grow into the agar with only slight surface growth.[13]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ PPLO Agar (Mycoplasma Agar)

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is medium amber, slightly opalescent.
Prepared Appearance:	Enriched with 30% Mycoplasma Supplement – Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.8 ± 0.2

Difco™ PPLO Broth (Mycoplasma Broth)

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 2.1% Solution at 25°C:	pH 7.8 ± 0.2

Difco™ Mycoplasma Supplement

Desiccated Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent.

Cultural Response

Difco™ PPLO Agar or PPLO Broth

Prepare the medium per label directions. Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate plates under 5-10% CO_2 at 35 ± 2°C for up to 7 days. Inoculate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate under 5-10% CO_2 at 35 ± 2°C for up to 7 days, then subculture (0.1 mL) to plates of the agar medium and incubate under 5-10% CO_2 at 35 ± 2°C for up to 7 days. Daily examine plates microscopically for growth.

ORGANISM	ATCC™	RECOVERY
Mycoplasma arginini	23243	Good
Mycoplasma bovis	25523	Good
Mycoplasma gallinarum	19708	Good

The base media are supplemented with Mycoplasma Supplement or Mycoplasma Enrichment w/o Penicillin because *Mycoplasma* spp. are fastidious in their growth requirements.[14]

Mycoplasma Supplement contains fresh yeast extract and horse serum. Yeast extract provides the preformed nucleic acid precursors that are required by *Mycoplasma* spp.[14] Horse serum supplies cholesterol, a growth stimulant.[14]

Mycoplasma Enrichment without Penicillin is a selective enrichment containing the inhibitor thallium acetate, to which a penicillin of choice (penicillin G or a broad-spectrum semisynthetic penicillin) can be added at the time of use to make it selective against gram-positive and gram-negative bacteria.

Identity Specifications

BBL™ Mycoplasma Agar Base (PPLO Agar Base)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.4% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to hazy.
Reaction of 3.4% Solution at 25°C:	pH 7.8 ± 0.2

BBL™ Mycoplasma Broth Base (PPLO Broth Base)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.0% solution, soluble in purified water upon warming. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.0% Solution at 25°C:	pH 7.8 ± 0.2

BBL™ Mycoplasma Broth Base (Frey)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.25% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.25% Solution at 25°C:	pH 7.7 ± 0.2

BBL™ Mycoplasma Enrichment without Penicillin

Reconstituted Appearance:	Dark brown, clear to trace hazy.

Cultural Response

BBL™ Mycoplasma Agar Base or Mycoplasma Broth Base

Prepare the medium per label directions (enriched with **BBL** Mycoplasma Enrichment without Penicillin). Inoculate agar plates with 0.1 mL of serial dilutions of the test organisms. Incubate for 7 days at 35 ± 2°C with CO_2 for *Mycoplasma pneumoniae* and anaerobically for *Mycoplasma orale*. Inoculate tubes of broth with 1.0 mL of serial dilutions of the test organisms and incubate for 7 days at 35 ± 2°C with 3-5% CO_2 for *M. pneumoniae* and anaerobically for *M. orale*. After 5 days of incubation, subculture tubes (0.1 mL) to Mycoplasma Agar and incubate at 35 ± 2°C for up to 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
Mycoplasma orale	23714	Good
Mycoplasma pneumoniae	1553	Good

BBL™ Mycoplasma Broth Base (Frey)

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 3-5% CO_2 for 7 days. Subculture to Mycoplasma Agar plates and incubate aerobically at 35 ± 2°C for 7 days. Examine plates microscopically for growth.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Growth in a dilution containing 10^3 CFU/mL
Mycoplasma gallisepticum	19610	Undiluted	Good
Mycoplasma synoviae	25204	Undiluted	Good

Formulae

Difco™ PPLO Agar

Approximate Formula* Per Liter

Beef Heart, Infusion from 50 g	6.0	g
Peptone	10.0	g
Sodium Chloride	5.0	g
Agar	14.0	g

Difco™ PPLO Broth

Consists of the the same ingredients without the agar.

BBL™ Mycoplasma Agar Base (PPLO Agar Base)

Approximate Formula* Per Liter

Beef Heart, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	7.0	g
Beef Extract	3.0	g
Yeast Extract	3.0	g
Sodium Chloride	5.0	g
Agar	14.0	g

BBL™ Mycoplasma Broth Base (PPLO Broth Base)

Consists of the same ingredients without the agar.

BBL™ Mycoplasma Broth Base (Frey)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	7.5	g
Papaic Digest of Soybean Meal	2.5	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Potassium Chloride	0.4	g
Magnesium Sulfate	0.2	g
Disodium Phosphate	1.6	g
Monopotassium Phosphate	0.1	g

Difco™ Mycoplasma Supplement

Approximate Formula* Per 30 mL Vial

Yeast Extract	0.01	g
Horse Serum, Desiccated	1.6	g

BBL™ Mycoplasma Enrichment without Penicillin

Approximate Formula* Per 30 mL Vial

Horse Serum	20.0	mL
Yeast Extract (fresh autolysate)	10.0	mL
Thallium Acetate	50.0	mg

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

Directions for Preparation from Dehydrated Product

Difco™ PPLO Agar
Difco™ PPLO Broth

1. **PPLO Agar:** Suspend 35 g of the powder in 700 mL of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
   PPLO Broth: Dissolve 21 g of the powder in 700 mL of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool medium to 50-60°C.
3. Aseptically add 300 mL **Difco** Mycoplasma Supplement to the medium. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Mycoplasma Agar Base
BBL™ Mycoplasma Broth Base

1. **Mycoplasma Agar Base:** Suspend 34 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
   Mycoplasma Broth Base: Suspend 20 g of the powder in 1 L of purified water. Mix thoroughly. Warm slightly to completely dissolve the powder.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C and add enrichment. Recommended enrichments include addition of 20 mL of horse serum and 5 mL of specially prepared yeast extract[11] to each 75 mL of cooled medium.
4. For a selective medium inhibitory to bacteria, add 30 mL of **BBL** Mycoplasma Enrichment without Penicillin to 70 mL of molten agar medium (50°C) or 70 mL of broth medium and add sterile penicillin G to a final concentration of 500 units/mL.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Mycoplasma Broth Base (Frey)

1. Dissolve 22.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C and add 100 mL of sterile inactivated horse serum. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Mycoplasma Supplement
BBL™ Mycoplasma Enrichment without Penicillin

1. Rehydrate with 30 mL of sterile purified water.
2. Rotate gently to dissolve.
3. Add 30 mL (the contents of one vial) to 70 mL of sterile medium base.
4. Dispense in plates or tubes as desired.

Procedure

Agar

Inoculate the surface of plates containing the complete medium by adding drops of liquid inoculum or by a swab-inoculation technique. Incubate plates at 35 ± 2°C for up to 21 days in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M. buccale*, *M. faucium*, *M. orale* or *M. salivarium* is suspected.[13]

Broth

Test material, either solid or liquid, should be directly inoculated into the broth medium. For preparation of stock organism suspensions, a block of agar culture can be added to the broth.

Following incubation at 35 ± 2°C in a moist aerobic atmosphere containing 5-10% carbon dioxide or anaerobically, if appropriate,[13] for various lengths of time, subculture aliquots of the broth to PPLO (Mycoplasma) Agar plates for visualization of typical colonies. The broth usually does not become turbid enough to confirm the presence of growth.

For a complete discussion of the isolation and identification of *Mycoplasma* spp. from clinical specimens, refer to appropriate procedures outlined in the references.[13-15]

Expected Results

Agar

PPLO colonies are round with a dense center and a less dense periphery, giving a "fried egg" appearance on PPLO (Mycoplasma) Agar. Vacuoles, large bodies characteristic of *Mycoplasma* spp., are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

Broth

After subculture to plates of PPLO (Mycoplasma) Agar, positive broth cultures produce colonies exhibiting the typical morphology; i.e., "fried egg" appearance.

Limitation of the Procedure

Thallium acetate can partially inhibit some mycoplasmas.[13]

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Availability

Difco™ PPLO Agar (Mycoplasma Agar)
Cat. No. 241210 Dehydrated – 500 g

BBL™ Mycoplasma Agar Base (PPLO Agar Base)
Cat. No. 211456 Dehydrated – 500 g

Difco™ PPLO Broth (Mycoplasma Broth)
Cat. No. 255420 Dehydrated – 500 g
255410 Dehydrated – 10 kg

BBL™ Mycoplasma Broth Base (PPLO Broth Base)
Cat. No. 211458 Dehydrated – 500 g

BBL™ Mycoplasma Broth Base (Frey)
Cat. No. 212346 Dehydrated – 500 g
212347 Dehydrated – 5 lb (2.3 kg)

Difco™ Mycoplasma Supplement
Cat. No. 283610 Vial – 6 x 30 mL*

BBL™ Mycoplasma Enrichment w/o Penicillin
Cat. No. 212292 Vial – 10 x 30 mL*

**Store at 2-8°C.*

Pagano Levin Base

Intended Use

Pagano Levin Base is used with TTC Solution 1% and neomycin in isolating and differentiating *Candida* spp.

Summary and Explanation

Pagano Levin Base as described by Pagano, Levin and Trejo[1] is selective for *Candida*. *Candida* spp. reduce TTC (2,3,5-triphenyltetrazolium chloride) in the medium to produce colonies with various degrees of color. Neomycin inhibits growth of most bacteria without appreciably influencing *Candida*. Gentamicin (50 µg/mL) may also be added to reduce bacterial populations according to Yamane and Saitoh.[2] Samaranayake, MacFarlane and Williamson[3] found that modified Pagano Levin Agar was far superior to the commonly used Sabouraud Dextrose Agar in detecting multiple yeast species in a single sample.

Principles of the Procedure

Peptone provides the carbon and nitrogen required for good growth of a wide variety of organisms. Yeast extract provides vitamins and cofactors. Dextrose is an energy source. Agar is the solidifying agent. TTC Solution 1%, added to the basal medium, facilitates the differentiation of yeast colonies based on the color change that occurs when a microorganism reduces TTC. Neomycin added to the base inhibits the growth of most bacteria.

User Quality Control

Identity Specifications

Difco™ Pagano Levin Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Light amber, slightly opalescent. With TTC and antibiotic – Light amber, milky.
Reaction of 6.6% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Difco™ Pagano Levin Base

Prepare the medium per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Candida albicans	26790	~10^7	Good	Cream to light pink
Candida albicans	36232	~10^7	Good	Pink to light red
Candida krusei	34135	~10^7	Good	White to light pink, spreading
Escherichia coli	25922	~10^8	Inhibition	–