Expected Results

H. influenzae produces colorless to gray, transparent, moist colonies with a characteristic "mousy" odor. *N. gonorrhoeae* produces small, translucent, raised, moist, colorless to grayish-white colonies.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

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6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Casman Agar Base

Cat. No. 211106 Dehydrated 500 g

Cetrimide Agar Base • Pseudosel™ Agar

Intended Use

Cetrimide (**Pseudosel**) Agar is used for the selective isolation and identification of *Pseudomonas aeruginosa*.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

King et al. developed Medium A (Tech Agar) for the enhancement of pyocyanin production by *Pseudomonas*.[4] Cetrimide (**Pseudosel**) Agar has the formula for Tech Agar but is modified by the addition of cetrimide (cetyl trimethyl ammonium bromide) for the selective inhibition of organisms other than *P. aeruginosa*.[5]

In 1951, Lowbury described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*.[5] Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.[6] Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.[7]

Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology[8] and the characteristic grapelike odor of aminoacetophenone.[9] *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide (**Pseudosel**) Agar, therefore, is a valuable culture medium in the identification of this organism.

Cetrimide (**Pseudosel**) Agar is widely recommended for use in the examination of cosmetics,[10] clinical specimens[8,11] for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.[12] It is also used in the microbiological examination of nonsterile pharmaceutical products for *Pseudomonas aeruginosa*.[1]

Principles of the Procedure

Gelatin peptone supplies the nutrients necessary to support growth. The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium.[13] Cetrimide is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*. Agar is a solidifying agent. Cetrimide Agar Base is supplemented with 1% glycerol as a source of carbon.

Formula

Difco™ Cetrimide Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Cetrimide (Tetradecyltrimethylammonium Bromide)	0.3	g
Agar	13.6	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45.3 g of the powder in 1 L of purified water containing 10 mL of glycerol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates in an inverted position (agar side up) at 35 ± 2°C for 18-48 hours.

Inoculate tubes with either pure cultures or with specimen material. Incubate tubes at 35 ± 2°C for 18-24 hours in an aerobic atmosphere.

Refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and tests for isolating *Pseudomonas aeruginosa* using Cetrimide Agar.[1]

Expected Results

Colonies that are surrounded by a blue-green pigment and fluoresce under short wavelength (254 nm) ultraviolet light may be presumptively identified as *Pseudomonas aeruginosa*. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light. Most non-*Pseudomonas*

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™ Cetrimide Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.53% solution with 1% glycerol, soluble in purified water upon boiling. Solution is light amber, opalescent, with a precipitate.
Prepared Appearance:	Light amber, opalescent, with precipitate.
Reaction of 4.53% Solution with 1% glycerol at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Cetrimide Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Incubate plates with *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	10^3- 2 × 10^3	Inhibition	—
Pseudomonas aeruginosa	27853	10^3	Good	Yellow-green to blue
Staphylococcus aureus	25923	10^3- 2 × 10^3	Inhibition	—
Pseudomonas aeruginosa	9027	10-100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

Identity Specifications

BBL™ Pseudosel™ Agar (prepared)

Appearance:	Cream to cream-tan and trace hazy to hazy.
Reaction at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ Pseudosel™ Agar (prepared)

Inoculate and incubate at 35-37°C for 18–48 hours. Incubate plates with *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-10^4	Inhibition
Pseudomonas aeruginosa	10145	10^3-10^4	Good
Stenotrophomonas maltophilia	13637	10^3-10^4	Inhibition
Pseudomonas aeruginosa	9027	10-100	Growth
Escherichia coli	8739	>100	No growth

Pseudosel™ Agar

species are inhibited, and some species of *Pseudomonas* may also be inhibited. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

Limitations of the Procedure

1. The type of peptone used in the base may affect pigment production.[7,14]
2. No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.
3. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescin production since this yellowing does not fluoresce.[7]
4. Some nonfermenters and some aerobic sporeformers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.[7]
5. Studies of Lowbury and Collins[6] showed *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.

References

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11. Forbes, Sahm, and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby Elsevier, St. Louis, Mo.
12. Horwitz, (ed). 2002. AOAC Official Method 955.13. Official methods of analysis of AOAC International, 17th ed, vol. 1, Rev. 1. AOAC International, Gaithersburg, Md.
13. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
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Availability

Difco™ Cetrimide Agar Base

AOAC BAM BS12 CCAM EP JP MCM9 USP

Cat. No. 285420 Dehydrated – 500 g†

BBL™ Pseudosel™ Agar

AOAC BAM BS12 CCAM EP JP MCM9 USP

United States and Canada

Cat. No. 297882 Prepared Plates – Pkg. of 10*†
221344 Tubed Slants – Pkg. of 10
221345 Tubed Slants – Ctn. of 100

Europe

Cat. No. 254419 Prepared Plates – Pkg. of 20*†

Mexico (Cetrimide Agar)

Cat. No. 252626 Prepared Plates (60 × 15 mm-style) – Pkg. of 10*†
257506 Prepared Plates – Pkg. of 10*†

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.

Chapman Stone Medium

Intended Use

Chapman Stone Medium is used for isolating and differentiating staphylococci based on mannitol fermentation and gelatinase activity.

Summary and Explanation

Chapman Stone Medium is prepared according to the formula described by Chapman.[1] It is similar to Staphylococcus Medium 110, previously described by Chapman,[2] except that the sodium chloride concentration is reduced to 5.5% and ammonium sulfate is included in the formulation. The inclusion of ammonium sulfate in the medium negates the need to add a reagent after growth has been obtained in order to detect gelatinase activity by Stone's method. Chapman Stone Medium is especially recommended for suspected food poisoning studies involving *Staphylococcus*.[3] It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation and the presence or absence of gelatin liquefaction.

Principles of the Procedure

Yeast extract and peptone provide nitrogen, carbon, sulfur, vitamins and trace nutrients essential for growth. Gelatin serves as a substrate for gelatinase activity. Ammonium sulfate allows detection of gelatin hydrolysis. D-Mannitol is the fermentable carbohydrate. Sodium chloride acts as a selective agent because most bacterial species are inhibited by the high salt content. Dipotassium phosphate provides buffering capability. Agar is the solidifying agent.

Formula

Difco™ Chapman Stone Medium

Approximate Formula* Per Liter		
Yeast Extract	2.5	g
Pancreatic Digest of Casein	10.0	g
Gelatin	30.0	g
D-Mannitol	10.0	g
Sodium Chloride	55.0	g
Ammonium Sulfate	75.0	g
Dipotassium Phosphate	5.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 20.2 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes. Omit autoclaving if used within 12 hours.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Chapman Stone Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a tendency to cake.
Solution:	20.2% solution, soluble in purified water upon boiling. Solution is light amber, opalescent with a precipitate.
Prepared Appearance:	Light to medium amber, opalescent with a precipitate.
Reaction of 20.2% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Chapman Stone Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours. Add bromcresol purple indicator to determine mannitol fermentation (yellow = positive).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO (GELATINASE)	MANNITOL FERMENTATION
Escherichia coli	25922	10^2-10^3	Inhibition	–	–
Staphylococcus aureus	25923	10^2-10^3	Good	+	+
Staphylococcus epidermidis	12228	10^2-10^3	Good	+	–