CETRIMIDE AGAR (7222)

Intended Use
Cetrimide Agar is used in the isolation and identification of *Pseudomonas aeruginosa*.

Product Summary and Explanation
Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.[1] This organism is a significant cause of burn and nosocomial infections.[2] The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.[1]

Pseudomonas aeruginosa produces a number of water-soluble pigments, including the yellow-green or yellow-brown fluorescent pigment pyoverdin (fluorescein).[2] When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *Pseudomonas aeruginosa* is created.[2] Agar containing Cetrimide has been used successfully to isolate *Pseudomononas aeruginosa* from contaminated specimens.[3]

King, Ward, and Raney developed Medium A (Tech Agar) to enhance the production of pyocyanin in *Pseudomonas* spp. [4] Cetrimide Agar is prepared according to this formula with the addition of Cetrimide.[4] Cetrimide Agar is recommended in the examination of food and in United States Pharmacopeia (USP XXIII) for use in Microbial Limit Test.[5,6]

Principles of the Procedure
Enzymatic Digest of Gelatin provides the nitrogen, vitamins, and carbon in Cetrimide Agar. Magnesium Chloride and Potassium Chloride enhance the production of pyocyanin and fluorescein.[7] Cetrimide (cetyltrimethylammonium bromide) is the selective agent. Cetrimide acts as a quaternary ammonium cationic detergent causing nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa*. Agar is the solidifying agent. Glycerol is supplemented as a source of carbon.

Formula / Liter	
Enzymatic Digest of Gelatin	20 g
Magnesium Chloride	1.4 g
Potassium Chloride	10 g
Cetrimide (Cetyltrimethylammonium Bromide)	0.3 g
Agar	13.6 g

Supplement /Liter
Glycerol.....10 mL

Final pH: 7.2 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 45.3 g of the medium and 10 mL of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is light to moderately hazy and grey-white w/precipitate.

Expected Cultural Response: Cultural response on Cetrimide Agar incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions / Colonies
Escherichia coli ATCC® 25922	10^3	Inhibited	--
Pseudomonas aeruginosa ATCC® 10145	10 - 300	Growth	Yellow-green to Blue-green
Pseudomonas aeruginosa ATCC® 27853	10 - 300	Growth	Yellow- green to Green
Staphylococcus aureus ATCC® 25923	10^3	Inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate *Pseudomonas aeruginosa* colonies directly on Cetrimide Agar by the streak method from nonselective medium or the clinical specimen. When plating directly from the specimen, the inoculum level will vary.

Results

Examine plates or tubes for the presence of characteristic blue, blue-green, or yellow-green pigment. *Pseudomonas aeruginosa* typically produces both pyocyanin and fluorescein.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescein production because this yellowing does not fluoresce.[4]
2. Some nonfermenters and some aerobic spores formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.[4]
3. Studies of Lowbury and Collins[7] showed *Ps. aeruginosa* can lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
4. Further tests are necessary for confirmation of *Ps. aeruginosa*.

Packaging

Cetrimide Agar	**Code No.**	**7222A**	**500 g**
		7222B	**2 kg**
		7222C	**10 kg**

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
2. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.)., Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **Robin, T., and J. M. Janda. 1984.** Enhanced recovery of *Pseudomonas aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect. Dis. **2**:207.
4. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
7. **Lowbury, E. J. L., and A. G. Collins.** 1955. The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa*. J. Clin. Pathol. **8**:47.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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