CETRIMIDE SELECTIVE AGAR

INTENDED USE
Remel Cetrimide Selective Agar is a solid medium recommended for use in qualitative procedures for selective isolation and presumptive identification of *Pseudomonas aeruginosa* and other non-fermenting, gram-negative bacilli.

SUMMARY AND EXPLANATION
King et al. developed a medium called Tech Agar which was used to enhance pyocyanin production by *Pseudomonas*.[1] In 1955, Lowbury described the use of cetrimide in a selective medium for *P. aeruginosa*.[2] Cetrimide Agar has the formula of Tech Agar, but is modified by the addition of cetrimide.

PRINCIPLE
P. aeruginosa is characterized by production of pyocyanin, a blue-green, water-soluble, nonfluorescent phenazine pigment. Potassium sulfate and magnesium chloride stimulate the production of pyocyanin and fluorescein. An ultraviolet light is used to visualize fluorescein production. Cetrimide (N-acetyl-NNN-trimethyl-ammonium bromide, cetavlon) inhibits bacteria other than *P. aeruginosa*, by causing nitrogen and phosphorus to be released from bacterial cells.

REAGENTS (CLASSICAL FORMULA)*
Gelatin Peptone	20.0 g	Cetrimide	0.3 g
Potassium Sulfate	10.0 g	Glycerol	10.0 ml
Magnesium Chloride	1.4 g	Agar	13.6 g
		Demineralized Water	1000.0 ml

pH 7.2 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory. Selective and nonselective media should be inoculated to increase the potential for recovery of gram-negative organisms present in low numbers and to provide for isolation of other organisms present in the specimen.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate Cetrimide Agar in ambient air at 33-37°C for 18-24 hours. **Note:** If slants are inoculated, tubes should be incubated with caps loosened.
4. Examine for typical colony morphology and characteristic blue-green pigmentation surrounding the growth. Using a long wave ultraviolet light (254 nm), examine colonies for yellow-green fluorescence. If the medium shows no typical colonies, re-incubate for another 24 hours.
5. Suspect colonies require confirmation by additional biochemical testing.

INTERPRETATION OF THE TEST
Pyocyanin Production:
Positive Test - Blue-green to yellow-green pigmentation surrounding growth
Negative Test - No color development

Fluorescein Production (requires the use of ultraviolet light):
Positive Test - Yellow-green fluorescence
Negative Test - No fluorescence

QUALITY CONTROL
All lot numbers of Cetrimide 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
Pseudomonas paraeruginosa ATCC® 9027	Ambient, 18-24 h @ 33-37°C	Growth, pyocyanin positive, fluorescein positive
Pseudomonas aeruginosa ATCC® 27853	Ambient, 18-24 h @ 33-37°C	Growth, pyocyanin positive, fluorescein positive
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
E. coli ATCC® 8739	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. Some enteric gram-negative bacilli may exhibit growth on Cetrimide Selective Agar and produce slight yellowing of the medium. This yellow color is easily distinguished from fluorescein by its lack of fluorescence.[3]
2. Of the pseudomonads, only *P. aeruginosa* is known to excrete pyocyanin as well as produce pyorubin simultaneously with pyocyanin and/or fluorescein. Pyorubin is a pink to red or dark maroon pigment.[3]
3. Occasional strains of *P. aeruginosa* may fail to produce pyocyanin.[3]
4. *P. aeruginosa* may lose its fluorescence under ultraviolet light if the cultures are left at room temperature for a short time. Fluorescence reappears after plates are reincubated.[3]
5. Cetrimide Selective Agar is both selective and differential. As such, low levels of the target organism may not be recoverable when inoculated directly onto this medium. Consult appropriate references to determine methods for recovery optimization.[3]

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BIBLIOGRAPHY

1. King, E.O., M.K. Ward, and D.E. Raney. 1954. J. Lab. Clin. Med. 44:301-307.
2. Lowbury, E.J. and A.G. Collins. 1955. J. Clin. Pathol. 8:47-48.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

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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