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*.

SUMMARY AND EXPLANATION
King et al. devised a medium called Tech Agar which was used to enhance pyocyanin production by *Pseudomonas*.[1] In 1951 and 1955, Lowbury described the use of cetrimide in a selective medium for *P. aeruginosa*.[2,3] The formula of Cetrimide Selective Agar is identical to that of Tech Agar with one exception, it is modified by addition of cetrimide. Cetrimide Selective Agar is formulated in conformance with harmonized *United States Pharmacopeia* (USP)/*European Pharmacopeia* (EP) guidelines.[4]

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 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.

PREPARATION OF DEHYDRATED CULTURE MEDIUM
1. Suspend 45.3 g of medium in 1000 ml of demineralized water.
2. Add 10 ml of glycerol.
3. Heat to boiling with agitation to completely dissolve.
4. Sterilize by autoclaving at 121°C for 15 minutes or according to established laboratory procedures.
5. Dispense into appropriate containers.

PROCEDURE
1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, and testing.[4,7]

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

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

QUALITY CONTROL
Each lot number of Cetrimide Selective Agar has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds harmonized USP/EP guidelines for microbial limits testing. Testing of control organisms should be performed in accordance with established laboratory quality control procedures.

CONTROL	INCUBATION	RESULTS
Pseudomonas aeruginosa ATCC® 9027	Aerobic, 18-24 h @ 30-35°C	Growth, blue-green pigmentation, yellow-green fluorescence
Pseudomonas aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 30-35°C	Growth, blue-green pigmentation, yellow-green fluorescence
Escherichia coli ATCC® 8739	Aerobic, 18-24 h @ 30-35°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 30-35°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.[5]
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.[5]
3. Occasional strains of *P. aeruginosa* may fail to produce pyocyanin.[5]
4. *P. aeruginosa* may lose its fluorescence under ultraviolet light if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.[5]
5. If Cetrimide Agar slants are inoculated, tubes should be incubated with caps loosened.[5]
6. 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.

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BIBLIOGRAPHY

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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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