SELLERS AGAR

INTENDED USE
Remel Sellers Agar is a solid medium recommended for use in qualitative procedures to differentiate and identify nonfermenting, gram-negative bacilli.

SUMMARY AND EXPLANATION
In 1964, Sellers designed a medium to distinguish between *Pseudomonas aeruginosa, Acinetobacter anitratus,* and *Acinetobacter lwoffii* all of which fail to produce acid reactions on Kligler Iron Agar or Triple Sugar Iron Agar.[1] Sellers' medium was formulated to differentiate nonfermenting, gram-negative bacilli by their ability to oxidize dextrose to acid in the presence of a high peptone concentration, produce nitrogen gas, fluoresce under ultraviolet light, and grow under anaerobic conditions in the presence of nitrate, thus changing the pH of the medium. Bhagwat and King evaluated this medium and confirmed the results of Sellers.[2]

PRINCIPLE
Peptone supplies carbon, nitrogen, and minerals. Yeast extract is a source of B-complex vitamins. Dextrose, added just prior to inoculation, diffuses into the medium during incubation. Oxidation of dextrose is indicated by a yellow band at the junction (interface) of the slant and butt. Magnesium sulfate and D-mannitol stimulate fluorescence by some organisms. Nitrogen gas production is stimulated by dipotassium phosphate and demonstrated by breaks, bubbles, or separations of the agar from the sides of the tube. Sodium nitrate and nitrite are substrates for the production of nitrogen gas by organisms capable of denitrification. Brom thymol blue and phenol red serve as pH indicators.

REAGENTS (CLASSICAL FORMULA)*

Peptone	20.0 g	Sodium Nitrate	1.0 g
D-Mannitol	2.0 g	Yeast Extract	1.0 g
Sodium Chloride	2.0 g	Sodium Nitrite	0.35 g
Magnesium Sulfate	1.5 g	Brom Thymol Blue	0.04 g
Dipotassium Phosphate	1.0 g	Phenol Red	8.0mg
L-Arginine	1.0 g	Agar	15.0 g
		Demineralized Water	1000.0 ml

pH 6.7 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Just prior to inoculation, add 0.15 ml (2 drops) of sterile 50% aqueous dextrose by allowing it to run down the side of the tube opposite the slant.
2. Using an inoculating needle, inoculate Sellers Agar from a pure, 18-24 hour culture growing on an agar plate. Stab the butt then streak back and forth across the entire surface of the agar slant.
3. Incubate aerobically with caps loosened at 33-37°C for 24-48 hours.

INTERPRETATION OF THE TEST
Slant (Fluorescein Production):
Positive Test - Yellow-green fluorescence under longwave ultraviolet light in the dark
Negative Test - No fluorescence

Slant/Butt Junction (Glucose Oxidation):
Positive Test - Yellow band formation at the interface of the slant and the butt
Negative Test - No yellow band

Butt (Arginine Dihydrolase):
Positive Test - Blue color (alkaline pH), anaerobic growth in the presence of nitrates
Negative Test - Green color

Gas Production (Denitrification):
Positive Test - Gas bubbles, splitting of medium, or displacement of medium from sides of tube
Negative Test - No gas production

QUALITY CONTROL
All lot numbers of Sellers 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 aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 33-37°C	Green slant, blue butt, no yellow band at slant/butt junction, (+) fluorescence, (+) gas
Acinetobacter baumannii ATCC® 19606	Aerobic, 18-24 h @ 33-37°C	Blue slant, green butt, yellow band at slant/butt junction, (-) fluorescence, (-) gas

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LIMITATIONS

1. The performance of Sellers Agar depends on proper inoculation to prevent a delay in the initiation of growth. The inoculum should not be taken from a broth suspension.[3]
2. Some strains of *P. aeruginosa* may require 48 hours to alkalinize the butt to a blue color.[3]
3. The yellow band at the slant/butt interface may disappear after 24 hours incubation due to oxidative depletion of glucose and a reversion back to alkalinity.[3]
4. *P. aeruginosa* usually oxidizes glucose, producing an acid reaction. It does not do so in Sellers Agar due to the presence of arginine and a high peptone concentration.[1] The alkali produced from the peptone breakdown neutralizes the acid.[4]
5. Some strains of *P. aeruginosa* do not produce nitrogen gas or fluoresce under ultraviolet light.[5]

BIBLIOGRAPHY

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4. Liu, P. 1952. J. Bacteriol. 64:773-781.
5. Gilardi, G.L. 1972. Am. J. Med. Technol. 38:65-72.

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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