CITRATE AGAR (SIMMONS)

INTENDED USE
Remel Citrate Agar (Simmons) is a solid medium recommended for use in qualitative procedures to differentiate gram-negative bacteria on the basis of citrate utilization.

SUMMARY AND EXPLANATION
In 1923, Koser developed a liquid medium to differentiate *Escherichia coli* and *Enterobacter aerogenes.*[1] The medium contained a single nitrogen source supplied by an inorganic ammonium salt and a single carbon source supplied by sodium citrate. In 1926, Simmons modified Koser's formulation by adding 1.5% agar and brom thymol blue indicator.[2]

PRINCIPLE
Organisms which use ammonium dihydrogen phosphate as a sole nitrogen source and sodium citrate as a sole carbon source will grow on Citrate Agar (Simmons). Such organisms extract nitrogen from ammonium salts in the medium, breaking it down to form ammonia. This leads to alkalization of the medium which causes the indicator, brom thymol blue, to change from green to blue. The rise in pH is due to an oxidative reaction which requires the tube caps to be loosened during incubation to ensure an adequate oxygen supply.

REAGENTS (CLASSICAL FORMULA)*

Sodium Chloride	5.0	g	Magnesium Sulfate	0.2	g
Sodium Citrate	2.0	g	Brom Thymol Blue	0.08	g
Ammonium Dihydrogen Phosphate	1.0	g	Agar	15.0	g
Dipotassium Phosphate	1.0	g	Demineralized Water	1000.0	ml

pH 6.9 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on proper inoculation. Lightly inoculate the slant from a single colony of an 18-24 hour culture or from a pure subculture of the test isolate.
2. Streak the surface of the slant, only.
3. Incubate in ambient air with caps loosened at 33-37°C for up to 4 days.
4. Examine daily for growth and an intense blue-green color development.

INTERPRETATION OF THE TEST
Positive Test - Growth with an intense blue-green color on the slant
Negative Test - No growth to trace growth with the slant remaining dark green

QUALITY CONTROL
All lot numbers of Citrate Agar (Simmons) 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
Enterobacter aerogenes ATCC® 13048	Ambient, 18-24 h @ 33-37°C	Positive
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Negative

LIMITATIONS
1. When inoculating a battery of biochemicals from a test isolate, either flame the loop before streaking the Citrate Agar (Simmons) slant or inoculate the slant first. Carryover of nutrients from other media may result in citrate being metabolized, indicating a false-positive reaction.[3]
2. If results are equivocal (+/-; e.g., *Providencia*), inoculate a new slant and incubate at room temperature for up to 7 days.[3]
3. A large inoculum may result in a yellow to tan color on the slant which should not be considered positive for citrate utilization.[3]
4. Growth without color change indicates that both carbon and nitrogen have been assimilated and the organism has entered the log phase. A blue color usually develops with an additional 24 hours of incubation.[3]

BIBLIOGRAPHY

1. Koser, S.A. 1923. J. Bacteriol. 8:493.
2. Simmons, J.S. 1926. J. Infect. Dis. 39:209.
3. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott 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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