SIMMONS CITRATE AGAR

INTENDED USE
Remel Simmons Citrate Agar 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 Simmons Citrate Agar. 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 occurs in the presence of oxygen. During incubation the tube caps must be kept loosened.

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.

PRECAUTIONS
This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

PREPARATION OF DEHYDRATED CULTURE MEDIUM
1. Suspend 24.2 g of medium in 1000 ml of demineralized water.
2. Heat to boiling with agitation to completely dissolve.
3. Dispense into appropriate containers and sterilize by autoclaving at 121°C for 15 minutes.
4. Allow medium to cool in a slanted position for use as slants.

PROCEDURE
1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, testing, and interpretation.

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
Each lot number of Simmons Citrate 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. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample 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 Simmons Citrate Agar 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, sample collection, storage and transportation, materials required, quality control, and limitations.

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