BBL™ Simmons Citrate Agar Slants

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Simmons Citrate Agar is a culture medium for the differentiation of gram-negative bacteria on the basis of citrate utilization.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using an inoculating needle, lightly inoculate tubes by streaking the slant and stabbing the butt with 18- to 24-h **Trypticase™** Soy Agar slant cultures.
   b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase** Soy Agar slants as nonselective controls for both organisms.
2. Examine tubes after 48 and 96 h for growth and color change.
3. Expected Results

Organisms	ATCC™	Recovery	Reaction
**Enterobacter aerogenes*	13048	Growth	Blue color of slant
**Escherichia coli*	25922	No growth to trace growth	No color change

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25°C and 30 – 35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Simmons Citrate Agar is used for the differentiation of gram-negative bacteria on the basis of citrate utilization.

V SUMMARY AND EXPLANATION

Koser,[1] in 1923, developed a liquid medium consisting of inorganic salts in which an ammonium salt was the only source of nitrogen and citrate was the sole carbon source in order to differentiate between what are now known as *Escherichia coli* and *Enterobacter aerogenes* as part of the IMViC (Indole-Methyl Red-Voges Proskauer-Citrate) reactions. Simmons,[2] in 1926, modified Koser's formulation with the addition of 1.5% agar and bromthymol blue.[3] Organisms capable of metabolizing citrate grow well on this medium.

VI PRINCIPLES OF THE PROCEDURE

Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon respectively will grow on this medium and produce an alkaline reaction as evidenced by a change in the color of the bromthymol blue indicator from green (neutral) to blue (alkaline).

VII REAGENTS

Simmons Citrate Agar

Approximate Formula* Per Liter Purified Water

Ingredient	Amount
Ammonium Dihydrogen Phosphate	1.0 g
Dipotassium Phosphate	1.0 g
Sodium Chloride	5.0 g
Sodium Citrate	2.0 g
Magnesium Sulfate	0.2 g
Agar	15.0 g
Bromthymol Blue	0.08 g

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[4,5] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Simmons Citrate Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Inoculate slants with growth from a pure culture using a light inoculum. Incubate all tubes for 24–48 h or up to 4 days at 35 ± 2°C in an aerobic atmosphere.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

A positive reaction is indicated by growth with an intense blue color in the slant. A negative reaction is evidenced by no growth to trace growth with no change in color (medium remains dark green).

Consult appropriate texts for additional differentiating characteristics.[6,7]

XI PERFORMANCE CHARACTERISTICS

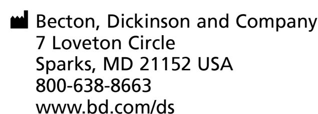
Prior to release, all lots of Simmons Citrate Agar slants are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase** Soy Agar cultures of *Escherichia coli* (ATCC 25922) and *Enterobacter aerogenes* (ATCC 13048) by streaking the slant and stabbing the butt with an inoculating needle. The tubes are read after 2 and 4 days incubation at 35 ± 2°C. *E. aerogenes* exhibits at least light growth accompanied by an alkaline (blue) change of the indicator in the medium. No reaction (color change) is evident with *E. coli* and growth may be completely inhibited to fair.

XII AVAILABILITY

Cat. No.	Description
221026	**BBL™** Simmons Citrate Agar Slants, Pkg. of 10 size K tubes
221027	**BBL™** Simmons Citrate Agar Slants, Ctn. of 100 size K tubes

XIII REFERENCES

1. Koser, S.A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. *8*:493-520.
2. Simmons, J.S. 1926. A culture medium for differentiating organisms of typhoid-colon-aerogenes groups and for isolation of certain fungi. J. Infect. Dis. *39*:209-214.
3. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
5. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
6. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
7. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.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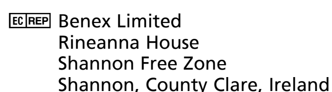
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