Expected Results

Cultures on Seven H11 Agar should be read within 5-7 days after incubation and once a week thereafter for up to 8 weeks.

Record Observations:[8]

1. Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7 days. Slow growers require more than 7 days for mature colony forms.
2. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.

Test all nonchromogenic mycobacteria on Seven H11 Agar with Aspartic Acid and Sodium Pyruvate for niacin production; only the rough nonchromogenic strains need to be tested for niacin. A culture must have at least 50-100 colonies with growth 3-4 weeks old. *M. tuberculosis* and the more rare *M. simiae* are usually niacin positive. Most other mycobacteria are niacin negative.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should be handled as directed for exchange of CO_2.

References

1. Cohn, Waggoner and McClatchy. 1968. Am. Rev. Respir. Dis. *98*:295.
2. Metchock, Nolte and Wallace. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Mitchison, Allen, Carrol, Dickinson and Aber. 1972. J. Med. Mycol. *5*:165.
4. McClatchy, Waggoner, Kanes, Cernich and Bolton. 1976. Am. J. Clin. Pathol. *65*:412.
5. Kilburn, Stottmeier and Kubica. 1968. Am. J. Clin. Pathol. *50*:582.
6. Garrod and O'Grady. 1971. Antibiotics and chemotherapy, 3rd ed. Williams & Wilkins, Baltimore, Md.
7. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Insitutes of Health. 1999. Biosafety in microbiology and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
8. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS, Centers for Disease Control, Atlanta, Ga.
9. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. Cernoch, Enns, Saubolle and Wallace. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., Weissfeld. American Society for Microbiology, Washington, D.C.
11. Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.

Availability

Difco™ Mycobacteria 7H11 Agar

Cat. No. 283810 Dehydrated – 500 g

BBL™ Seven H11 Agar Base

Cat. No. 212203 Dehydrated – 500 g

BBL™ Seven H11 Agar

BS10 CMPH MCM7

Cat. No. 221870 Prepared Plates (Deep Fill) – Pkg. of 10*
221391 Prepared Slants (A Tubes) – Pkg. of 10*
221392 Prepared Slants (A Tubes) – Ctn. of 100*
296105 Prepared Slants (C Tubes) – Pkg. of 10*
297704 Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Selective Seven H11 Agar

BS10 CMPH MCM7

Cat. No. 221868 Prepared Plates (Deep-fill) – Pkg. of 10*
297315 Prepared Slants (A Tubes) – Pkg. of 10*
297639 Prepared Slants (A Tubes) – Ctn. of 100*
297184 Prepared Slants (C Tubes) – Pkg. of 10*
297654 Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Seven H11 Agar with Aspartic Acid and Sodium Pyruvate

Cat. No. 221958 Prepared Slants (A Tubes) – Pkg. of 10*

BBL™ Middlebrook 7H11 Agar//Selective 7H11 Agar

BS10 CMPH MCM7

Cat. No. 297250 Prepared Bi-Plate Dishes – Pkg. of 20*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

BBL™ Taxo™ TB Niacin Test Strips and Control

Cat. No. 231741 Vial – 25 strips*
231735 Cartridge, Control – 50 discs*

**Store at 2-8°C.*

Simmons Citrate Agar

Intended Use

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

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.

Principles of the Procedure

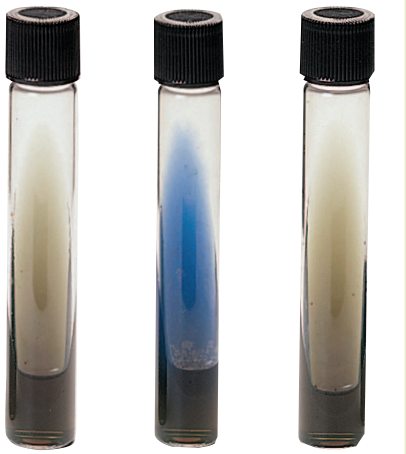
Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon,

User Quality Control

Identity Specifications

BBL™ Simmons Citrate Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many dark and gray flecks.
Solution:	2.42% solution, soluble in purified water upon boiling. Solution is medium to dark, green, clear to slightly hazy.
Prepared Appearance:	Medium to dark, green, clear to slightly hazy, with a small amount of precipitate and as many as a large amount of insolubles.
Reaction of 2.42% Solution at 25°C:	pH 6.9 ± 0.2



Uninoculated Tube | *Enterobacter aerogenes* ATCC™ 13048 | *Escherichia coli* ATCC™ 25922

Cultural Response

BBL™ Simmons Citrate Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 4 days.

ORGANISM	ATCC™	RECOVERY	REACTION
Enterobacter aerogenes	13048	Good	Alkaline (blue)
Escherichia coli	25922	Partial to complete inhibition	–
Klebsiella pneumoniae	33495	Good	Alkaline (blue)
Shigella flexneri	9199	Complete inhibition	–

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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).

Formula

BBL™ Simmons Citrate Agar

Approximate Formula* Per Liter

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.*

Directions for Preparation from Dehydrated Product

1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Allow to cool in a slanted position for use as slants. The agar also may be used as a plating medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

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

Expected 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.[4,5]

References

1. Koser. 1923. J. Bacteriol. *8*:493.
2. Simmons. 1926. J. Infect. Dis. *39*:209.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
5. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Simmons Citrate Agar

AOAC | BAM | CCAM | COMPF | ISO | USDA

Cat. No.	211620	Dehydrated – 500 g
	221026	Prepared Slants – Pkg. of 10*
	221027	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*