

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, 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. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

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

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
<i>Enterobacter aerogenes</i>	13048	Good	Alkaline (blue)
<i>Escherichia coli</i>	25922	Partial to complete inhibition	—
<i>Klebsiella pneumoniae</i>	33495	Good	Alkaline (blue)
<i>Shigella flexneri</i>	9199	Complete inhibition	—



Availability

BBL™ Simmons Citrate Agar

AOAC BAM CCAM COMPF ISO

Skim Milk

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

*Store at 2-8°C.

Skim Milk • Skim Milk Medium

Intended Use

Skim Milk is used for preparing microbiological culture media. Skim Milk Medium may be used for the cultivation and differentiation of microorganisms based on the coagulation and proteolysis of casein.

Summary and Explanation

Skim Milk is soluble, spray-dried skim milk. When prepared in a 10% solution, it is equivalent to fresh skim milk. Skim Milk can be used to prepare Skim Milk Agar for detecting proteolytic microorganisms in foods,¹ including dairy products.² It can also be used to prepare litmus milk, a differential test medium for determining lactose fermentation and for detecting proteolytic enzymes that hydrolyze casein (milk protein) and cause coagulation (clot formation).³

Skim Milk Medium, 10% skim milk solution prepared in tubes, is used for the maintenance and propagation of lactic acid bacteria. It is especially useful in species differentiation within the genus *Clostridium*.

Principles of the Procedure

Skim Milk is a source of lactose and casein and other nutrients required for the growth of lactobacilli.⁴ Clostridial species can be differentiated based on their ability to enzymatically degrade proteins to peptones (peptonization) or coagulate milk.⁵ It may be used to detect the stormy fermentation produced by *Clostridium perfringens*.

User Quality Control

Identity Specifications

Difco™ Skim Milk

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	10% solution, soluble in purified water upon warming. Solution is white, opalescent.
Prepared Appearance:	Off-white to beige, opaque.
Reaction of 10% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Difco™ Skim Milk

Prepare the medium per label directions. Inoculate with a drop or loopful of fresh culture and incubate at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC™	GROWTH	APPEARANCE
<i>Clostridium perfringens</i>	12919	Good	Stormy fermentation
<i>Escherichia coli</i>	25922	Good	Acid, curd
<i>Lactobacillus rhamnosus</i>	9595	Good	Acid, curd

Formula

Difco™ Skim Milk

Approximate Formula* Per Liter

Skim Milk Powder 100.0 g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 100 g of the powder in 1 L of purified water.
2. Warm, if necessary, to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Heat the medium in a boiling water bath for 2-5 minutes with caps loosened and cool to room temperature with caps tightened.

Inoculate tubes using a calibrated loop or sterile disposable pipet. For the study of anaerobic organisms, sterile mineral oil can be layered over the medium following inoculation.

Incubate tubes, with tightened caps for clostridia and loosened caps for other organisms, at 35 ± 2°C and read at intervals for 7 days for growth and reactions.

Expected Results

Consult an appropriate reference for the expected reactions for specific microbial species.^{4,5}

Limitation of the Procedure

Skim Milk Medium supports growth of many microorganisms. Perform microscopic examination and other biochemical tests to identify isolates to the genus and species level, if necessary.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
2. Wehr and Frank (ed.). 2004. Standard methods for the examination of the dairy products, 17th ed. American Public Health Association, Washington, D.C.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Sneath and Holt (ed.). 1986. Bergey's Manual™ of systematic bacteriology, vol.2. Williams & Wilkins, Baltimore, Md.
5. Allen, Emery and Siders. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Skim Milk

Cat. No. 232100 Dehydrated – 500 g

BBL™ Skim Milk Medium

Cat. No. 298240 Prepared Tubes (D Tubes) – Pkg. of 10