

## SIMMONS CITRATE AGAR (7156)

### Intended Use

**Simmons Citrate Agar** is used for the differentiation of microorganisms on the basis of citrate utilization.

### Product Summary and Explanation

Koser first developed a liquid medium for differentiating coliforms from fecal coliforms. Fecal coliforms were unable to use citrate as the sole source of carbon and inorganic ammonium salt as a sole source of nitrogen. Non-fecal coliforms, such as *Enterobacter aerogenes* could use citrate in such a medium with resultant alkalinity. Liquid medium had the disadvantage of appearing turbid when a large inoculum was used, although no growth had taken place. This observation led Simmons to devise a solid medium that eliminated the problem with turbidity.<sup>2</sup>

Simmons Citrate Agar is a modification of Koser's medium, with the addition of bromthymol blue and 1.5% agar. Organisms able to metabolize citrate grow luxuriantly. The medium is alkalized and changes from green to deep blue in 24 – 48 hours. *Escherichia coli* either do not grow at all on this medium, or grow so sparsely that no change in reaction is apparent. Simmons Citrate Agar is recommended for differentiation of enteric Gram-negative bacilli from clinical specimens,<sup>3,4</sup> water samples,<sup>5</sup> and food samples.<sup>6,7</sup>

### Principles of the Procedure

Ammonium Dihydrogen Phosphate is the sole source of nitrogen in Simmons Citrate Agar. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the sole source of carbon in this medium. Magnesium Sulfate is a cofactor for a variety of metabolic reactions. Bromthymol Blue is the pH indicator. Organisms that can utilize Ammonium Dihydrogen Phosphate and Sodium Citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline). Agar is the solidifying agent.

### Formula / Liter

Ammonium Dihydrogen Phosphate.....	1 g
Dipotassium Phosphate .....	1 g
Sodium Chloride.....	5 g
Sodium Citrate.....	2 g
Magnesium Sulfate.....	0.2 g
Bromthymol Blue .....	0.08 g
Agar .....	15 g

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

### Directions

1. Suspend 24.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and yellow with green tinge to green with yellow tinge.

**Prepared Appearance:** Prepared medium is forest green and trace to slight hazy.

**Expected Cultural Response:** Cultural response on Simmons Citrate Agar incubated at the appropriate atmosphere and temperature and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions
<i>Enterobacter aerogenes</i> ATCC® 13048	Direct inoculation	Growth	Blue medium
<i>Escherichia coli</i> ATCC® 25922	Direct inoculation	Partial to full inhibition	Green medium
<i>Salmonella choleraesuis</i> ATCC® 13076	Direct inoculation	Growth	Blue medium
<i>Salmonella typhimurium</i> ATCC® 14028	Direct inoculation	Growth	Blue medium
<i>Shigella flexneri</i> ATCC® 12022	Direct inoculation	Partial to full inhibition	Green medium

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

1. Obtain a pure colony of the test organism.
2. Streak only the surface of the slant with a light inoculum.
3. Incubate tubes at 35 ± 2°C for 18 – 48 hours with loose caps.

### Results

A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by inhibition to poor growth without change in color (medium remains green).

### Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

### Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Some citrate positive organisms require a 48 hour incubation or longer for a pH change to occur.<sup>8</sup>

### Packaging

Simmons Citrate Agar	Code No.	7156A	500 g
		7156B	2 kg
		7156C	10 kg

### References

1. Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. 8:493.
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.
3. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Baron, E. J., L. R. Peterson, S. M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9<sup>th</sup> ed. Mosby-Year Book, Inc., St. Louis, MO.
5. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1995. Standard methods for the examination of water and wastewater, 19<sup>th</sup> ed. American Public Health Association, Washington, D.C.
6. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
7. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm).
8. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.