# Simmons Citrate Agar, Granulated

**GM099**

Simmons Citrate Agar, granulated is recommended for differentiating the members of *Enterobacteriaceae* on the basis of citrate utilization.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Magnesium sulphate | 0.200 |
| Ammonium dihydrogen phosphate | 1.000 |
| Dipotassium phosphate | 1.000 |
| Sodium citrate | 2.000 |
| Sodium chloride | 5.000 |
| Bromothymol blue | 0.080 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 6.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Cool to 45-50°C. Mix well and distribute in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Precaution:Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour,if the above precaution is ignored.

## Principle And Interpretation

These media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser (1) containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons (2) modified Kosers formulation by adding agar and bromo thymol blue (3). It is recommended by APHA (4).

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

## Quality Control

**Appearance**
Cream to yellow coloured granular medium

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Forest green coloured slightly opalescent gel forms in tubes as slants

**Reaction**
Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2

**pH**
6.60-7.00

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

| Organism | Inoculum (CFU) | Growth | Citrate utilization |
|---|---|---|---|

**Cultural Response**

| | | | |
|---|---|---|---|
| *Enterobacter aerogenes ATCC 13048* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Escherichia coli ATCC 25922* | >=$10^3$ | inhibited | |
| *Salmonella* Choleraesuis *ATCC 12011* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Salmonella* Enteritidis *ATCC 13076* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Salmonella* Typhi *ATCC 6539* | 50-100 | fair-good | negative reaction, green colour |
| *Salmonella* Typhimurium *ATCC 14028* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Shigella dysenteriae ATCC 13313* | >=$10^3$ | inhibited | |

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, 1926, J. Infect. Dis., 39:209.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. Rice E.W., Baird R.B., Eaton A. D., and Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd Ed., APHA, Washington, D.C.

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