



**SIMMONS CITRATE AGAR (BIS)**

**TM 1291**

**INTENDED USE**

For differentiation between faecal coliforms and members of the aerogenes group on the basis of citrate utilization

**COMPOSITION**

Ingredients	Gms/Ltr.
Agar	15.000
Sodium chloride	5.000
Sodium citrate	2.000
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.200
Bromo thymol blue	0.080

**PRODUCT SUMMARY AND EXPLANATION**

Simmons Citrate Agar is a synthetic test agar proposed by Simmons (1926) for the identification of microorganisms (particularly of enteric Gram-negative bacilli from clinical specimens) on the basis of their metabolism of citrate, being the sole carbohydrate source. The media is 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 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 modified Koser's formulation by adding agar and bromothymol blue. It is recommended by APHA. It is also recommended by BIS for isolation of *Escherichia coli*.

**PRINCIPLE**

Ammonium dihydrogen phosphate and sodium citrate serves as the sole nitrogen and carbon source respectively. Bromo thymol blue is the pH indicator. Dipotassium hydrogen phosphate acts as a buffer. Sodium chloride maintains the osmotic balance. Metabolism of citrate leads to alkalization of the medium, which is indicated by a change in the color of the pH indicator bromothymol blue from green to deep blue. Magnesium sulfate is a cofactor for a variety of metabolic reactions.

**INSTRUCTION FOR USE**

1. Dissolve 24.28 grams in 1000 ml distilled water.



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## PRODUCT DATA SHEET

2. Gently heat to boiling to dissolve the medium completely.
3. Mix well and distribute in tubes.
4. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
5. Cool the tubes as slants.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder:** Yellow colour, homogeneous free flowing powder

**Appearance of prepared medium:** Forest green colour, slightly opalescent gel

**pH (at 25°C) :** 6.8 ± 0.2

### INTERPRETATION:

Culture characteristics observed after inoculating 50-100 CFU, for incubation period of 18 - 24 hours at 35 ± 2°C.

Microorganisms	ATCC	Inoculum (CFU)	Growth	Citrate utilization, medium colour changes
<i>Enterobacter aerogenes</i>	13048	50-100	Luxuriant	Positive, blue colour
<i>Salmonella enteritidis</i>	13076	50-100	Luxuriant	Positive, blue colour
<i>Salmonella typhimurium</i>	14028	50-100	Luxuriant	Positive, blue colour
<i>Salmonella typhi</i>	6539	50-100	Fair to Good	Negative, green colour
<i>Shigella dysenteriae</i>	13313	≥ 1000	Inhibited	-----
<i>Escherichia coli</i>	25922	≥ 1000	Inhibited	-----

### STORAGE & STABILITY

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

### REFERENCES

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, J.S. (1926). A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolating of certain fungi. J. Infect. Dis. 39: 209-241.



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3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. American Public Health Association, 1981, Standard Methods for the Examination of Water and Wastewater, 15th ed., APHA Inc., Washington, D.C.
5. Bureau of Indian Standards, IS:5887 (Part II) 1976, reaffirmed 1986



**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.