

## TM 348 - SIMMONS CITRATE AGAR

### INTENDED USE

For differentiation of *Enterobacteriaceae* on the basis of citrate utilization.

### PRODUCT SUMMARY AND EXPLANATION

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 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.

### COMPOSITION

Ingredients	Gms / Ltr
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

### PRINCIPLE

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.

### INSTRUCTION FOR USE

- Dissolve 24.28 grams in 1000 ml purified/ distilled water.
  - Heat, to boiling, to dissolve the medium completely.
  - Mix well and distribute in tubes or flasks.
  - Sterilize by autoclaving at 15 psi 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.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.  
**Appearance of prepared medium** : Forest green coloured slightly opalescent gel forms in tubes as slants.  
**pH (at 25°C)** : 6.8±0.2

### INTERPRETATION

Cultural characteristics observed after an incubation.



Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Citrate utilization	Incubation Temperature	Incubation Period
<i>Klebsiella aerogenes</i>	13048	50-100	Good-luxuriant	Positive reaction, blue colour	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	$\geq 10^3$	Inhibited	-	35-37°C	18-24 Hours
<i>Salmonella Typhi</i>	6539	50-100	Fair-good	Negative reaction, green colour	35-37°C	18-24 Hours
<i>Salmonella Typhimurium</i>	14028	50-100	Good-luxuriant	Positive reaction, blue colour	35-37°C	18-24 Hours
<i>Shigella dysenteriae</i>	13313	$\geq 10^3$	Inhibited	-	35-37°C	18-24 Hours
<i>Salmonella Choleraesuis</i>	12011	50-100	Good-luxuriant	Positive reaction, blue colour	35-37°C	18-24 Hours
<i>Salmonella Enteritidis</i>	13076	50-100	Good-luxuriant	Positive reaction, blue colour	35-37°C	18-24 Hours

**PACKAGING:**

In pack size of 100 gm and 500 gm bottles.

**STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°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.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

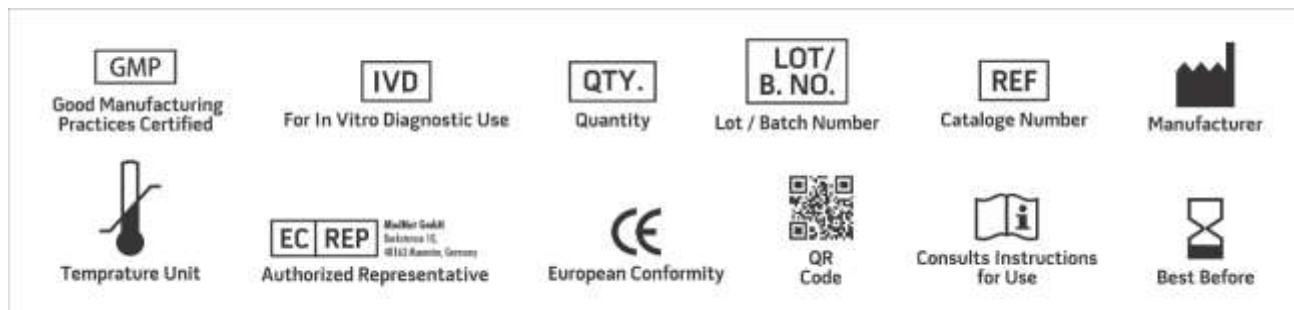
**DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.



**REFERENCES**

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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. Second Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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8. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
9. Simmons, 1926, J. Infect. Dis., 39:209.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.



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

**\*For Lab Use Only**  
**Revision: 08 Nov., 2019**