# Simmons Citrate Agar

**M099**

## Intended Use:

Simmons Citrate Agar is recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization from clinical and non clinical samples. .

## 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. Mix well and distribute in tubes or flasks. 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 (6) 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 (9) modified Kosers formulation by adding agar and bromothymol blue (7). It is recommended by APHA (3).

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.

## Type of specimen

Pure isolate from clinical and non clinical samples.

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1.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.
2. The pH affects the performance of the medium and must be correctly monitored.

## Performance and Evaluation

Performace of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

**Appearance**
Cream to yellow homogeneous free flowing powder

**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.

**Cultural Response**

| Organism | Inoculum (CFU) | Growth | Citrate utilisation |
|---|---|---|---|
| **Cultural Response** | | | |
| *# Klebsiella aerogenes ATCC 13048 (00175*)* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Escherichia coli ATCC 25922 (00013*)* | >=$10^3$ | inhibited | |
| *Salmonella Typhi ATCC 6539* | 50-100 | fair-good | negative reaction, green colour |
| *Salmonella Typhimurium ATCC 14028 (00031*)* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Shigella dysenteriae ATCC 13313* | >=$10^3$ | inhibited | |
| *Salmonella Choleraesuis ATCC 12011* | 50-100 | good-luxuriant | positive reaction, blue colour |
| *Salmonella Enteritidis ATCC 13076 (00030*)* | 50-100 | good-luxuriant | positive reaction, blue colour |

Key: * Corresponding WDCM numbers
# Formerly known as *Enterobacter aerogenes*

## Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

## Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd 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.
6. Koser, 1923, J. Bact., 8:493.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 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.

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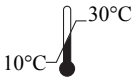
IVD — In vitro diagnostic medical device

CE Marking




Storage temperature

Do not use if package is damaged

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