Solid medium used for the differentiation between members of *Enterobacteriaceae* family based on the utilization of citrate as the only source of carbon.

**Simmon’s Citrate Agar**

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| REF: LV.1/SC01.100.0100 100 gram REF: LV.1/SC01.250.0250 250 gram |  REF: LV.1/SC01.500.0500 500 gram  |

# CLINICAL SIGNIFICANCE

Citrate utilization test is commonly employed as part of a group of tests, the IMViC (Indole, Methyl Red, VP and Citrate) tests that distinguish between members of the *Enterobacteriaceae* family based on their metabolic by-products. Citrate utilization can be used to differentiate between coliforms such as *Klebsiella* *aerogenes* (Positive Citrate utilization) which occur naturally in the soil and in aquatic environments from fecal coliforms such as *Escherchia coli (*Negative Citrate utilization*)* whose presence would indicate fecal contamination.

# METHOD PRINCIPLE

# Ammonium Dihydrogen Phosphate is the sole source of nitrogen. 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. Agar is the solidifying agent. Organisms capable of utilizing ammonium dihydrogen phosphate and citrate will grow unrestricted on this medium. If citrate can be used, the microbe will accumulate alkaline/basic byproducts. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6.

# MEDIA COMPOSITION

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| **Item**  | **Formula in g/L**  |
| Magnesium sulphateAmmonium dihydrogen phosphateDipotassium phosphate Sodium citrateSodium chlorideBromothymol blueAgar  | 0.211250.0815 |

## pH 6.8 ± 0.2 at 25°C

# PRECAUTIONS AND WARNINGS (2)

Media to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses,..).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.
* Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Simmon’s citrate Agar

material safety data sheet.

# MEDIA PREPARATION, STORAGE AND STABILITY

**Lab.Vie**. Simmon’s citrate Agar should be stored between 10-30°C in a firmly 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 avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

##  PROCEDURE

1. Suspend 24.28 grams in 1 liter distilled water.
2. Adjust pH to pH 6.8 ± 0.2 at 25°C.
3. Heat, to boiling, to dissolve the medium completely
4. . Mix well and distribute in appropriate containers.
5. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

## Deterioration

**Lab.Vie**. Simmon’s Citrate Agar is cream to yellow cream to yellow homogeneous free flowing powder. Prepared Media is forest green in color. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

**SPECIMEN COLLECTION AND PRESERVATION**

# For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

# TYPE OF SPECIMEN

# Isolated microorganisms from clinical and non-clinical samples (food and water samples)

# EQUIPMENT REQUIRED NOT PROVIDED

# Sterile cups

# Sterile petri-dishes

# Incubator

# Autoclave

# QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

# PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU’s).

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| **Microorganism** | **Result** | **Citrate Utilization** |
| *Citrobacter freundii ATCC 43864* | Luxuriant growth | Positive and gives blue color |
| *Klebsiella (Enterobacter)**aerogenes (ATCC 13048)* | Luxuriant growth | Positive and gives blue color |
| *Salmonella Typhi**ATCC 6539* | Fair-good growth | Negative and gives green color |
| *Salmonella Typhimurium ATCC 14028* | Luxuriant growth | Positive and gives blue color |
| *Shigella dysenteriae**ATCC 13313* | Inhibited | - |
| *Escherichia coli**ATCC 25922* | Inhibited | - |
| *Salmonella Enteritidis**ATCC 13076* | Luxuriant growth | Positive and gives blue color |

# REFERENCES

1. Simmons, 1926, J. Infect. Dis., 39:209.
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3. 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.
4. MacFaddin, J. D. 1985. Media for isolation-cultivation-identificationmaintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/Bacteriolo gicalAnalyticalmanualBAM/default.htm.
6. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

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| **SYMBOLS IN PRODUCT LABELLING**  |
|   IVD For in-vitro diagnostic use  |   Number of <n> test in the pack  |
|  LOT Batch Code/Lot number  | A black and white triangle with a exclamation mark  Description automatically generated Caution  |
|  REF Catalogue Number  | Do not use if package is damaged   |
|   Temperature Limitation   Expiration Date   Manufactured by  |  Consult Instruction for use      |

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