Simmons Citrate Agar

**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**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.080</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**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.

**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.

**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 (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(7)

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**

Performance 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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Citrate utilisation</th>
</tr>
</thead>
<tbody>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blue colour</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>fair-good</td>
<td>negative reaction, green colour</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blue colour</td>
</tr>
<tr>
<td>Shigella dysenteriae ATCC 1313</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>Salmonella Choleraesuis ATCC 12011</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blue colour</td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC 50-100 (00030*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blue colour</td>
</tr>
</tbody>
</table>

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 (8,9).
Reference

In vitro diagnostic medical device

CE Marking

Storage temperature

10°C - 30°C

Do not use if package is damaged

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner4u.eu

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.