Christensen Citrate Sulphite Agar, w/ 1.5% Agar

**Intended Use:**
Recommended for differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production in accordance with FDA BAM, 1998.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>0.200</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.500</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.100</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.400</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>0.080</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 25.29 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Cool in a slanted position to give slants with generous butts.

**Principle And Interpretation**
Christensen Citrate Sulphite Agar, w/1.5% agar is used for the differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production in accordance with FDA BAM, 1998(1). Christensen Citrate Sulphite Agar was formulated by Edwards and Ewing (2,3) as a modification of the Christensen Iron Agar (4). Christensen reported that all members of genera *Escherichia, Enterobacter, Citrobacter* and *Salmonella* as well as Alkalescens-Dispar were capable of utilizing citrate as a source of energy while *Shigella* species failed to utilize citrate. Organisms that metabolize citrate as a sole source of carbon cleave citrate to oxaloacetate and acetate via the citritase enzyme. Another enzyme, oxaloacetate decarboxylase, then converts oxaloacetate to pyruvate and CO$_2$. Further, this CO$_2$ combines with sodium and water to form sodium carbonate, an alkaline compound (5). As a result, the pH of medium rises and the indicator, phenol red changes from orange red to cerise. Presence of the cerise colour indicates a positive finding for citrate utilization.

Yeast extract provide the necessary nutrients mainly nitrogenous and vitamins for the growth of the organisms. L-Cysteine hydrochloride is a reducing agent. Dextrose is the fermentable carbohydrate. Sodium citrate is the energy source for citrate utilizing organisms. Care should be taken while inoculating, as, a too heavy inoculum may give a false positive result (6).

The reduction of ferric ammonium citrate to iron sulphide by H2S producing organisms is indicated by blackening of the medium. Sodium thiosulphate enhances H2S production. Strong positive cultures upon prolonged incubation turn the entire butt black.

According to FDA BAM, recovery of *Shigella* is done in two different ways. First is the conventional method wherein the organism is grown in a selective media such as *Shigella Broth Base* (M1326) with novobiocin, isolated in selective media such as *MacConkey Agar* (M081D) and further confirmed using biochemical tests. In the second method, *Shigella* is identified using DNA hybridization technology. In the conventional method, the organisms isolated in selective agar are confirmed using various biochemical reactions including citrate utilization test. For citrate utilization test, inoculate the isolated colonies into Christensen Citrate Sulphite Agar, w/1.5% agar (M495F). *Shigella* does not utilize citrate and give negative citrate utilization reaction.

**Type of specimen**
Food samples
Specimen Collection and Handling:
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
Read the label before opening the container. Wear protective gloves/protection 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
1. Care should be taken while inoculating, as, a too heavy inoculum may give a false positive result (1)
2. Other biochemical tests must be carried out in conjunction for confirmatory results

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
Light yellow to light pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Orange red coloured, very slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.53% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

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>
<th>H2S</th>
</tr>
</thead>
<tbody>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction, cerise colour</td>
<td>negative reaction, no colour change</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction, no colour change</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction, cerise colour</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC 50-100 13076 (00030*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction, cerise colour</td>
<td>positive reaction, blackening of medium</td>
</tr>
<tr>
<td>Klebsiella pneumoniae ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>weakly positive, orange-pink colour</td>
<td>negative reaction, no colour change</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Disclaimer:**

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**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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.

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 (7,8).

**Reference**