Christensen Citrate Sulphite Agar

Intended Use:
Recommended for differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulhide production.

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>14.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.7±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 24.29 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 12 to 15 lbs pressure (118 to 121°C) for 15 minutes. Cool the tubes in slanted position to give slants with generous butts.

Principle And Interpretation
Christensen Citrate Sulphite Agar is a modification of the Christensen Iron Agar (1). This modification was described by Edwards and Ewing (2). Christensen reported that all members of genera *Escherichia, Enterobacter, Citrobacter* and *Salmonella* as well as *Alkalascens-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 CO2. Further, this CO2 combines with sodium and water to form sodium carbonate, an alkaline compound (3). 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. Medium constituent yeast extract provide the necessary nutrients mainly nitrogenous and vitamins for the growth of the organisms.

L-Cysteine hydrochloride is a reducing agent. Dextrose (Glucose) 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 (4).

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. Some members of *Salmonella* like *Salmonella Typhi* are weakly positive and require 2-5 days for hydrogen sulphite production.

Type of specimen
Isolated Microorganisms from clinical and non clinical samples.

Specimen Collection and Handling
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1, 9, 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

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
1. Strong positive cultures upon prolonged incubation turn the entire butt black.
2. Some members of *Salmonella* like *Salmonella Typhi* are weakly positive and require 2-5 days for hydrogen sulphite production.
3. It is important not to carry over any nutrients into the citrate medium because this will result in false positive tests.

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 1.4% Agar gel.

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

Reaction
Reaction of 2.42% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

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>luxuriant</td>
<td></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>
<tr>
<td>Shigella flexneri ATCC 12022 (00126*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Shigella sonnei ATCC 25931 50-100</td>
<td>luxuriant</td>
<td></td>
<td>negative reaction, no colour change</td>
<td>negative reaction, no change</td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

# Formerly known as *Enterobacter aerogenes*
Storage and Shelf Life
Store between 10-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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