Kligler Iron Agar, Modified

Kligler Iron Agar, Modified is recommended for identification of *Yersinia enterocolitica*. It can also be used for the differential identification of gram-negative enteric bacilli on the basis of the fermentation of glucose (dextrose), lactose and H2S production.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>20.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Glucose anhydrous</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium thiosulphate pentahydrate</td>
<td>0.300</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 57.41 grams of dehydrated powder in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts.

Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

**Principle And Interpretation**

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1) and Russels Double Sugar Agar (2) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (3). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella Typhi* from other Salmonellae and also *Salmonella Paratyphi A* from *Salmonella Scottmulleri* and *Salmonella Enteritidis* (5). Kligler Iron Agar, Modified is used for the identification of *Yersinia enterocolitica*, as recommended by ISO Committee (6). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow.

Kligler Iron Agar, in addition to casein enzymic hydrolysate, beef and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of or ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates
the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

**Quality Control**

**Appearance**
Light yellow to pink homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Red coloured, clear to slightly opalescent gel forms in tubes as slants

**Reaction**
Reaction of 5.74% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**
7.20-7.60

**Cultural Response**
M078A: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Slant</th>
<th>Butt</th>
<th>Gas</th>
<th>H2S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acid reaction, yellowing of the medium</td>
<td>acid reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>negative reaction no blackening of medium</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em> ATCC 13047</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acid reaction, yellowing of the medium</td>
<td>acid reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>negative reaction no blackening of medium</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 13883</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, red colour of the medium</td>
<td>Acidic reaction, positive reaction</td>
<td></td>
<td>negative reaction no blackening of medium</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> ATCC 6380 50-100</td>
<td>luxuriant</td>
<td></td>
<td>alkaline reaction, red colour of the medium</td>
<td>acid reaction, yellowing of the medium</td>
<td>Negative reaction</td>
<td>Positive reaction,blackening of medium</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, red colour of the medium</td>
<td>acid reaction, negative reaction</td>
<td></td>
<td>negative reaction no blackening of medium</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 6539 50-100</td>
<td>luxuriant</td>
<td></td>
<td>alkaline reaction, red colour of the medium</td>
<td>acid reaction, negative reaction</td>
<td></td>
<td>positive reaction,blackening of medium</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, red colour of the medium</td>
<td>acid reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>negative reaction,blackening of medium</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> ATCC 12022</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, red colour of the medium</td>
<td>acid reaction, yellowing of the medium</td>
<td>negative reaction</td>
<td>negative reaction,blackening of medium</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Yersinia enterocolitica
ATCC 27729
50-100 luxuriant alkaline reaction, red colour of the medium acidic reaction, variable yellowing of the medium reaction
negative reaction, no blackening of medium

Enterobacter aerogenes
ATCC 13048
50-100 luxuriant acidic reaction, yellowing of the medium acidic reaction, positive yellowing of the medium reaction
negative reaction, no blackening of medium

Citrobacter freundii ATCC 8090
50-100 luxuriant acidic reaction, yellowing of the medium acidic reaction, positive yellowing of the medium reaction
positive reaction, blackening of medium

Salmonella Paratyphi A
ATCC 9150
50-100 luxuriant alkaline reaction, red colour of the medium acidic reaction, positive yellowing of the medium reaction
negative reaction, no blackening of medium

Salmonella Schottmuelleri
ATCC 10719
50-100 luxuriant alkaline reaction, red colour of the medium acidic reaction, positive yellowing of the medium reaction
positive reaction, blackening of medium

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

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