Kligler Iron Agar

Intended Use:
Recommended for the differential identification of gram-negative enteric bacilli from clinical and non clinical samples on the basis of the fermentation of dextrose, lactose and H₂S production.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>15.000</td>
</tr>
<tr>
<td>HM Peptone B #</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose(Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.024</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>

**Formula adjusted, standardized to suit performance parameters

# - Equivalent to Beef extract

Directions

Kligler Iron Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts / slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (9) and Russels Double Sugar Agar (7) 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 (3). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates Salmonella Typhi from other Salmonellae and also Salmonella Paratyphi A from Salmonella Scottmuelleri and Salmonella Enteritidis (4). 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 peptone, HM peptone B 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 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.

**Type of specimen**
Isolated microorganism from clinical, food, dairy and water samples.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,10,11).
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**
In Vitro diagnostic use. 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. Results should be noted after 18-24 hours. Else it might result in erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous 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 pink homogeneous free flowing powder

**Colour medium**
Red coloured

**Quantity of medium**
100 ml of medium in glass bottle.

**Reaction**
7.20-7.60

**Sterility test**
Passes release criteria

**Cultural Response**
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>Gas</th>
<th>H2S</th>
<th>Slant</th>
<th>Butt</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli ATCC 25922 (00013</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction</td>
<td>negative reaction, no blackening of medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes ATCC 13048 (00175</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction</td>
<td>negative reaction, no blackening of medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Citrobacter freundii ATCC 8090  50-100 luxuriant positive reaction positive reaction, blackening of medium acidic reaction, yellowing of the medium

Proteus vulgaris ATCC 6580  50-100 luxuriant negative reaction positive reaction, blackening of medium alkaline reaction, red colour of the medium acidic reaction, yellowing of the medium

Klebsiella pneumoniae ATCC 13883 (00087*)  50-100 luxuriant positive reaction negative reaction, no blackening of medium acidic reaction, yellowing of the medium acidic reaction, yellowing of the medium

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

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

Salmonella Typhi ATCC 6539  50-100 luxuriant negative reaction positive reaction, blackening of medium alkaline reaction, red colour of the medium acidic reaction, yellowing of the medium

Salmonella Enteritidis ATCC 13076 (00030*)  50-100 luxuriant positive reaction positive reaction, blackening of medium alkaline reaction, red colour of the medium acidic reaction, yellowing of the medium

Shigella flexneri ATCC 12022 (00126*)  50-100 luxuriant negative reaction negative reaction, no blackening of medium alkaline reaction, red colour of the medium acidic reaction, yellowing of the medium

Pseudomonas aeruginosa ATCC 27853 (00025*)  50-100 luxuriant negative reaction negative reaction, blackening of medium alkaline reaction, red colour of the medium acidic reaction, yellowing of the medium

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

Enterobacter cloacae ATCC 13047 (00083*)  50-100 luxuriant positive reaction negative reaction, no blackening of medium acidic reaction, yellowing of the medium acidic reaction, yellowing of the medium

Key:* Corresponding WDCM numbers

Storage and Shelf Life
On receipt store between 15-25°C. 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 (5,6).
Reference


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