**Formula adjusted, standardized to suit performance parameters**

**Directions**
Suspend 57.52 grams in 1000 ml purified/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 to 45-50ºC 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.

Note: Avoid overheating otherwise it may produce precipitate in the medium.

**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 HiVeg™ Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. Kligler Iron HiVeg™ Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other *Salmonella* 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 HiVeg™ Agar, in addition to HiVeg™ special peptone, HiVeg™ extract, HiVeg™ peptone No. 3 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.

**Intended Use:**
Recommended for differential identification of gram-negative enteric bacilli from clinical and non-clinical samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ special peptone</td>
<td>15.000</td>
</tr>
<tr>
<td>HiVeg™ extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>HiVeg™ peptone No. 3</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><strong>Final pH (at 25°C)</strong></td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Please refer disclaimer Overleaf.**
Pure cultures of suspected organisms from plating media such as MacConkey HiVeg™ Agar (MV081), Bismuth Sulphite HiVeg™ Agar (MV027) or Deoxycholate Citrate Agar, HiVeg™ (MV065); SS HiVeg™ Agar (MV108) etc. are inoculated on Kligler Iron HiVeg™ 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

**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 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**
7.20-7.60

**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</em> ATCC 25922 (00013*)</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</em> ATCC 13048 (00175*)</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>
### Citrobacter freundii ATCC 8090
- **50-100 luxuriant positive reaction**
- Positive reaction, blackening of medium
- Acidic reaction, yellowing of the medium

### Proteus vulgaris ATCC 6380
- **50-100 luxuriant negative reaction**
- Positive reaction, blackening of medium
- Alkaline reaction, red colour 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

### Proteus vulgaris ATCC 6380
- **50-100 luxuriant negative reaction**
- Positive reaction, blackening of medium
- Alkaline reaction, red colour of the medium

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### Proteus vulgaris ATCC 6380
- **50-100 luxuriant negative reaction**
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- Alkaline reaction, red colour 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

### Salmonella Paratyphi A ATCC 9150
- **50-100 luxuriant positive reaction**
- Negative reaction, no blackening of medium
- Alkaline reaction, red colour of the medium

### Salmonella Schottmuelleri ATCC 10719
- **50-100 luxuriant positive reaction**
- Positive reaction, blackening of 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

### Salmonella Enteritidis ATCC 13076 (00030*)
- **50-100 luxuriant positive reaction**
- Positive reaction, blackening of medium
- Acidic reaction, yellowing of the medium

### Shigella flexneri ATCC 12022 (00126*)
- **50-100 luxuriant negative reaction**
- Positive reaction, blackening of medium
- Alkaline reaction, red colour of the medium

### Pseudomonas aeruginosa ATCC 27853 (00025*)
- **50-100 luxuriant negative reaction**
- Positive reaction, blackening of medium
- Alkaline reaction, red colour 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

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

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**Key:**
- * Corresponding WDCM numbers
- (#) Formerly known as *Enterobacter aerogenes*

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**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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).
**Reference**


**Disclaimer :**

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