

Technical Information

Kligler Iron Agar

Product Code: DM 1078I

Application: - Kligler Iron Agar (DM1078I) is recommended for identification of Pseudomonas species. It can also be used for the differential identification of gram-negative enteric bacilli on the basis of the fermentation of glucose, lactose and H₂S production.

Composition**

| Ingredients | Gms / Litre |
|--|-------------|
| Beef extract | 3.000 |
| Yeast extract | 3.000 |
| Casein enzymic hydrolysate | 20.000 |
| Sodium chloride | 5.000 |
| Lactose | 10.000 |
| Glucose anhydrous | 1.000 |
| Ferrous ammonium sulphate, 6H ₂ O | 0.500 |
| Sodium thiosulphate pentahydrate | 0.500 |
| Phenol red | 0.025 |
| Agar | 15.000 |
| Final pH (25°C) | 7.4±0.2 |

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler⁽¹⁾ and Russels Double Sugar Agar⁽²⁾. This medium is used as a differentiation medium for typhoid, dysentery and allied bacilli⁽³⁾. Bailey and Lacey replaced phenol red for Andrade indicator 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 Scottmuelleri and Salmonella Enteritidis⁽⁵⁾. Kligler Iron Agar is also recommended by ISO Committee for identification of Pseudomonas species⁽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 makes the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which shows a colour change in response to acid produced during the fermentation of sugars. The combination of ferric ammonium sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is indicated 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 indicate 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 (DM1081), Bismuth Sulphite Agar (DM1027) or Deoxycholate Citrate Agar (DM1065), SS Agar (DM1108) etc. are inoculated on Kligler Iron Agar for identification.

Methodology

Suspend 57.70 grams of dehydrated powder in 1000 ml distilled water. Shake well & heat 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.

Quality Control

Physical Appearance

Light yellow to light 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 5.77% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH Range 7.20-7.60

Cultural Response/ characteristics

DM 1078I: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

| Organism | Inoculum (CFU) | Growth | Slant | Butt | Gas | H ₂ S |
|------------------------------------|----------------|-----------|---|--|-------------------|---|
| Escherichia coli ATCC 25922 | 50-100 | luxuriant | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium | positive reaction | negative reaction, no medium blackening of medium |
| Enterobacter cloacae ATCC 13047 | 50-100 | luxuriant | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium | positive reaction | negative reaction, no medium blackening of medium |
| Klebsiella pneumoniae ATCC 13883 | 50-100 | luxuriant | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium | positive reaction | negative reaction, no medium blackening of medium |
| Proteus vulgaris ATCC 6380 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | negative reaction | positive medium blackening of reaction |
| Pseudomonas aeruginosa ATCC 27853 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | negative reaction | negative reaction, no medium blackening of medium |
| Salmonella Typhi ATCC 6539 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | negative reaction | positive medium blackening of reaction |
| Salmonella Enteritidis ATCC 13076 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | positive reaction | positive medium blackening of reaction |
| Shigella flexneri ATCC 12022 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | negative reaction | negative reaction, no medium blackening of medium |
| Yersinia enterocolitica ATCC 27729 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | variable reaction | negative reaction, no medium blackening of medium |
| Enterobacter aerogenes ATCC 13048 | 50-100 | luxuriant | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium | positive reaction | negative reaction, no medium blackening of medium |
| Salmonella Paratyphi A | 50-100 | luxuriant | alkaline reaction, red | acidic | positive | negative |



| | | | | | | |
|--|--------|-----------|--|---|----------------------|---|
| ATCC 9150 | | | colour of the medium | reaction, yellowing of the medium | reaction | reaction,no medium blackening of medium |
| Salmonella Schottmuelleri ATCC 10719 | 50-100 | luxuriant | alkaline reaction, red colour of the medium | acidic reaction, yellowing of the medium | positive reaction | positive medium blackening of reaction |
| Citrobacter freundii ATCC 8090 | 50-100 | luxuriant | acidic reaction, yellowing of the medium | acidic reaction, yellowing of the medium | positive reaction | positive medium blackening of reaction |

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Russell F. F., 1911, J. Med. Res., 25:217.
2. Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.
3. Kligler I. J., 1918, J. Exp. Med., 28:3 19.
4. Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.
5. Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., N.Y.
6. International Organization for Standardization (ISO), 1995, Draft ISO/DIS 13720.

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- User must ensure suitability of the product(s) in their application prior to use.
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