

Technical Information

Kligler Iron Agar, Modified

Product Code: DM 1078A

Application: - 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**

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 sulphate	0.200
Sodium thiosulphate pentahydrate	0.300
Phenol red	0.025
Agar	15.000
Final pH (at 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⁾ and is used as a differentiation medium for diagnosis of typhoid, dysentery and allied bacilli ⁽³⁾. Bailey and Lacey substituted phenol red for previously used Andrade indicator 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⁾. Modification of this medium 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 ferrous sulphate and sodium thiosulphate helps in 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 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 (DM1081), Bismuth Sulphite Agar (DM1027) or Deoxycholate Citrate Agar (DM1065), SS Agar (DM1108) etc. are inoculated on Kligler Iron Agar for identification.

Methodology

Suspend 57.41 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 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 range 7.20-7.60

Cultural Response / characteristics

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

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H ₂ S
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
<i>Enterobacter cloacae</i> ATCC 13047	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	alkaline reaction, red colour of the the medium	alkaline reaction, red colour of the the medium	positive reaction	negative reaction no blackening of medium
<i>Proteus vulgaris</i> ATCC 6380	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	negative reaction	positive reaction, blackening of medium
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	negative reaction	negative reaction no blackening of medium
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	negative reaction	positive reaction, blackening of medium
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction, blackening of medium



<i>Shigella flexneri</i> ATCC 12022	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	negative reaction	negative reaction no blackening of medium
<i>Yersinia enterocolitica</i> ATCC 27729	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	negative reaction	negative reaction no blackening of medium
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction, blackening of medium
<i>Salmonella Paratyphi A</i> ATCC 9150	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
<i>Salmonella Schottmuelleri</i> ATCC 10719	50-100	luxuriant	alkaline reaction, red colour of the the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction, blackening of medium

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), 1994, Draft ISO/DIS 10273.

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