Technical Information

Kligler Iron Agar

Product Code: DM 1078

Application: - Kligler Iron Agar is recommended for the differential identification of gram-negative enteric bacilli on the basis of the fermentation of dextrose, lactose and H_2S production.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	15.000
Beef extract	3.000
Yeast extract	3.000
Proteose peptone	5.000
Lactose	10.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
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 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]. Fermentation of dextrose results in production of acid, which turn the indicator yellow from red. 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 turns the entire medium yellow.

Kligler Iron Agar, in addition to peptic digest of animal tissue, 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 ferrous 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 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 (DM1027) or Deoxycholate Citrate Agar (DM1065), SS Agar (DM1108) etc. are inoculated on Kligler Iron Agar for identification.

Methodology

Suspend 57.52 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Mix well and distribute into 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 1inch butts.

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

Cultural Response / characteristics

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

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H_2S
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
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 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
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 valgaris ATCC 6380	50-100	luxuriant	Alkaline reaction, red colour of the medium	alkaline reaction, red colour of the medium	negative reaction	Positive medium blackening of reaction,
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	Alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	Negative 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,

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
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
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 cloacae ATCC 13047	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction,no medium 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^{o} 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.

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