TM 142 - KLIGLER IRON AGAR, MODIFIED

INTENDED USE

For identification of *Yersinia enterocolitica* and for differential identification of gram-negative enteric bacilli based on dextrose and lactose fermentation and H_2S production.

PRODUCT SUMMARY AND EXPLANATION

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler and Russels Double Sugar Agar and is used as a differentiation medium for typhoid, dysentery and allied bacilli. Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator. Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other *Salmonella* and also *Salmonella* Paratyphi A form *Salmonella* Scottmuelleri and *Salmonella* Enteritidis. Kligler Iron Agar, modified is used for the identification of *Yersinia enterocolitica*, as recommended by ISO Committee. 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.
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 nor lactose. Gas production 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, Bismuth Sulphite Agar or Deoxycholate Citrate Agar, SS Agar etc. are inoculated on Kligler Iron Agar for identification.

COMPOSITION

Ingredients	Gms / Ltr
Beef extract	3.000
Yeast extract	3.000
Tryptone	20.000
Sodium chloride	5.000
Lactose	10.000
Dextrose (Glucose)	1.000
Ferrous sulphate	0.200
Sodium thiosulphate pentahydrate	0.300
Phenol red	0.025
Agar	15.000

PRINCIPLE

Kligler Iron Agar, in addition to tryptone, Beef extract 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 or 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.

INSTRUCTION FOR USE

- Dissolve 57.41 grams of dehydrated powder 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 psi pressure (121°C) for 15 minutes.
- Allow the tubes to cool to 45-50°C 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 SPECIFICATIONS

Appearance of Powder : Light yellow to pink homogeneous free flowing powder.
Appearance of prepared medium : Red coloured, clear to slightly opalescent gel forms in tubes as slants.
pH (at 25°C) : 7.4±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Gas	H_2S	Slant	Butt	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Luxuriant	Positive reaction	Negative reaction, no blackening of medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Klebsiella aerogenes	13048	50-100	Luxuriant	Positive reaction	Negative reaction, no blackening of medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Citrobacter freundii	8090	50-100	Luxuriant	Positive reaction	Positive reaction, blackening of medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Proteus vulgaris	6380	50-100	Luxuriant	Negative reaction	Positive reaction, blackening of medium	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Klebsiella pneumoniae	13883	50-100	Luxuriant	Positive reaction	Negative reaction, no blackening of medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Salmonella Paratyphi A	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	35 - 37°C	18-48 Hours

Salmonella Schottmuelleri	10719	50-100	Luxuriant	Positive reaction	Positive reaction, blackening of medium	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Salmonella Typhi	6539	50-100	Luxuriant	Negative reaction	Positive reaction, blackening of medium	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Salmonella Enteritidis	13076	50-100	Luxuriant	Positive reaction	positive reaction, blackening of medium	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Shigella flexneri	12022	50-100	Luxuriant	Negative reaction	Negative reaction, no blackening of medium	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours
Pseudomonas aeruginosa	27853	50-100	Luxuriant	Negative reaction	Negative reaction, no blackening of medium	Alkaline reaction, red colour of the medium	Alkaline reaction, red colour of the medium	35 - 37°C	18-48 Hours
Yersinia enterocolitica	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	35 - 37°C	18-48 Hours
Enterobacter cloacae	13047	50-100	Luxuriant	Positive reaction	Negative reaction, no blackening of medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35 - 37°C	18-48 Hours

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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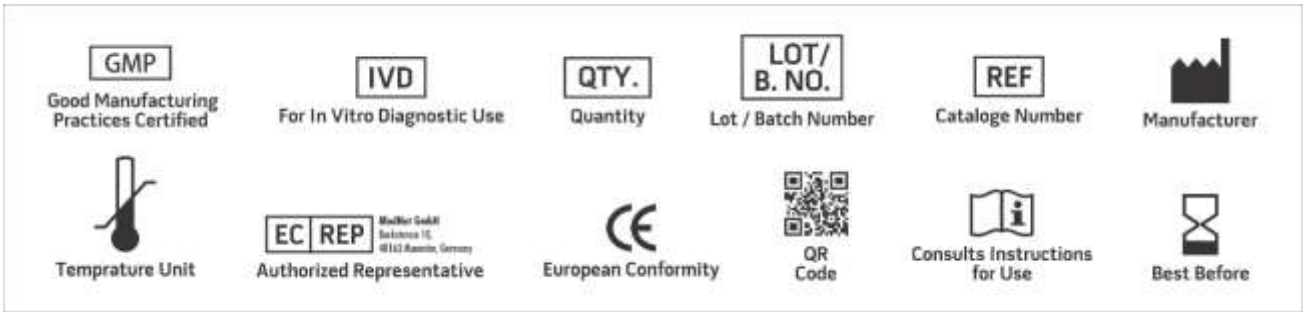

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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