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KLIGLER IRON AGAR TM 141

INTENDED USE

For the differentiation identification of gram-negative enteric bacilli based on fermentation of dextrose and lactose and production of H_2S.

COMPOSITION

Ingredients	Gms/Ltr.
Peptone	15.000
Agar	15.000
Lactose	10.000
Proteose peptone	5.000
Sodium chloride	5.000
Yeast extract	3.000
Beef extract	3.000
Dextrose	1.000
Sodium thiosulphate	0.300
Ferrous sulphate	0.200
Phenol red	0.0240

PRODUCT SUMMARY AND EXPLANATION

KLIGLER IRON AGAR is used for the differentiation identification of Gram-negative (*Enterobacteria*) based on fermentation of Dextrose, Lactose and H_2S production. Russell1 described a combination of two sugars in an agar medium to differentiate gram negative intestinal microorganisms. Kligler added lead acetate to Russell's medium and reported successful differentiation of typhoid paratyphoid group. Bailey and Lacy simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. Kligler Iron Agar differentiates the lactose fermenters from non-fermenters. It differentiates *Salmonella typhi* from other Salmonella species and also *Salmonella paratyphi* A from *Salmonella Scottmuelleri* and *Salmonella enteritidis*. Kligler Iron Agar is recommended for differentiation of enteric Gram negative bacilli from clinical specimens and food samples. It is recommended by Edward and Ewing for determination of H_2S production by enteric gram negative bacilli.

PRINCIPLE

The medium contains. Peptone, Proteose peptone, Yeast extract and Beef extract those provides nitrogenous, compounds, vitamins, minerals and amino acids sources to the medium. Agar is a solidifying agent. Incorporation of Dextrose and Lactose helps in differentiation of the coliforms based on their ability to ferment different carbohydrates, detected by the indicator Phenol red. 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. Since there is low concentration of Dextrose, acid production is very limited and therefore re-oxidation takes place. Dextrose fermentation results in production of acid, which turns the indicator from red to yellow. 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
The medium is recommended for the identification of colonies picked off from plating media such as MacConkey Agar, Bismuth Sulphite Agar, or Desoxycholate Citrate Agar, etc.

INSTRUCTION FOR USE

1. Dissolve 57.52 gms in 1000ml distilled water.
2. Gently heat to boiling with gentle swirling and dissolve the medium completely.
3. Distribute into test-tubes.
4. Sterilize by autoclaving at 15 psi (at 121°C) for 15 minutes.
5. Cool the tubes in slanting position with 1 inch butts.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder: Light yellow to pink colour, homogeneous free flowing powder.
Appearance of prepared medium: Orange to red colour, clear to slightly opalescent gel.
pH (at 25°C): 7.4 ± 0.2

INTERPRETATION:

Cultural characteristics observed after incubation at 35 ± 2°C for 18 - 24 hours.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H_2S
Escherichia coli	25922	50 - 100	Luxuriant	Acidic reaction, yellowing of	Acidic reaction, yellowing of	Positive reaction	Negative reaction, no blackening

				the medium	the medium		of medium
Proteus vulgaris	6380	50 – 100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction, blackening of medium
Shigella flexneri	12022	50 – 100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Negative reaction, no blackening of medium
Salmonella typhi	19430	50 – 100	Luxuriant	Alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	Negative reaction	Positive reaction, blackening of medium
Klebsiella pnuemoniae	13883	50 – 100	Luxuriant	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	Positive reaction	Negative reaction, no blackening of medium
Pseudomonas aeruginosa	27853	50 – 100	Luxuriant	Alkaline reaction, red colour of the medium	Alkaline reaction, red colour of the medium	Negative reaction	Negative reaction, no blackening of medium

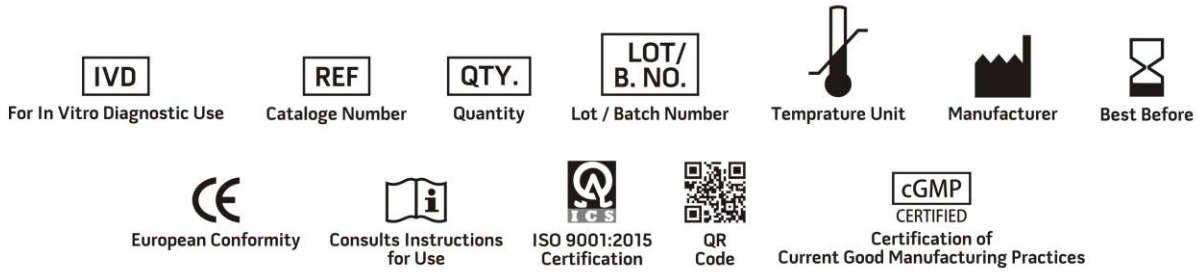
STORAGE & STABILITY

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°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.

REFERENCES

1. Russell, F. F. 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. 25:217
2. Kligler, I. J. 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health 7:1042- 1044
3. Kligler, I. J. 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. 28:319-322
4. Bailey, S. F., and L. R. Lacy. 1927. A modification of the Kligler lead acetate medium. J. Bacteriol. 13:183

5. Ewing, Edwards and Ewings, Identification of the Enterobacteriaceae, 4th ed, Elsevier Science Publishing Co., Inc., N.Y. (1986).
6. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
7. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.



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