TM 1553- KLIGLER IRON AGAR (ISO 13720-1995, ISO 10273:2003)

INTENDED USE

For identification of *Pseudomonas* species 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 differential medium used for distinguishing lactose fermenters from the non-fermenters. It is also recommended by the ISO committee for the identification of *Pseudomonas* species. This medium is a combination of the lead acetate medium described by Kligler and Russels Double sugar agar. The medium utilizes dextrose and lactose fermentation capacity of the organism along with their ability to produce H_2S to create differentiation among them.

COMPOSITION

Ingredients	Gms / Ltr
Casein enzymatic hydrolysate	20.000
Agar	15.000
Lactose	10.000
Sodium chloride	5.000
Yeast extract	3.000
Beef extract	3.000
Dextrose	1.000
Sodium thiosulphate pentahydrate	0.500
Ferrous ammonium sulphate, $6H_2O$	0.500
Phenol red	0.025

PRINCIPLE

The medium contains Casein enzymatic hydrolysate, Yeast extract and Beef extract which 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. 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. The combination of Ferrous sulphate and Sodium thiosulphate enables the detection of hydrogen sulphide production, which is evidenced 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 Dextrose. When 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 enough acid is produced in the slant, due to fermentation of Lactose, to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates neither the fermentation of 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.

INSTRUCTION FOR USE

- Dissolve 58.02 grams in 1000ml distilled water.
- Gently heat to boiling with gentle swirling and dissolve the medium completely.
- Distribute into test tubes.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Allow the tubes to cool to cool to 45-50°C in slanted position to form slopes with about 1-inch butts.

Note: Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder	:	Light yellow to pink, Homogeneous free flowing powder
Appearance of Prepared medium	:	Red coloured, clear to slightly opalescent gel
pH (at 25°C)	:	7.4± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with addition of TTC solution 1% (TS 042).

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Slant	Butt	Gas	H_2S production
Escherichia coli	25922	50-100	Luxuriant	++	++	+	--
Salmonella typhi	19430	50-100	Luxuriant	+++	++	-	++++
Pseudomonas aeruginosa	27853	50-100	Luxuriant	+++	++	-	--
Klebsiella pnuemoniae	13883	50-100	Luxuriant	++	++	+	--
Proteus vulgaris	6382	50-100	Luxuriant	+++	++	-	++++
Salmonella enteritidis	13076	50-100	Luxuriant	+++	++	+	++++
Shigella flexneri	12022	50-100	Luxuriant	+++	++	-	--
Klebsiella aerogenes	13048	50-100	Luxuriant	++	++	+	--

+ = Positive reaction
- = Negative Reaction
++= Acidic reaction, Yellowing of the medium
+++= Alkaline reaction, Red colour of the medium
++++ = Positive reaction, Blackening of the medium
-- = Negative Reaction, No blackening of medium

PACKAGING

In 500 gm packaging size.

STORAGE

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.

Product Deterioration: Do not use powder, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

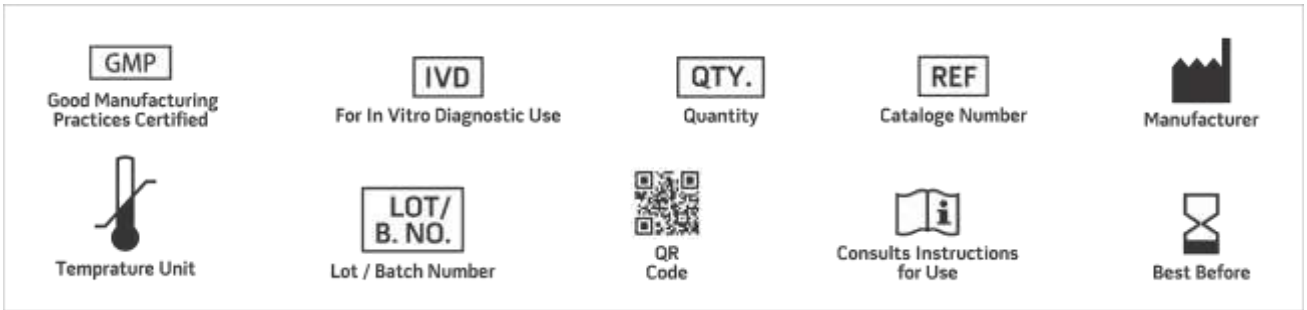
DISPOSAL

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

REFERENCES

1. Russell F.F., J. Med. Res., 25:217. (1911).
2. Kligler I.J., Am. J. Publ. Health, 7:1042. (1917).
3. Kligler J.J., J. Exp. Med., 28:319. (1918).
4. Bailey S.F. and Lacey G.R., J. Bact., 13:182. (1927).

5. Ewing, Edwards and Ewings, Identification of the Enterobacteriaceae, 4th ed, Elsevier Science Publishing Co., Inc., N.Y. (1986).
6. International Organization for Standardization (ISO). Draft ISO/DIS 13720.



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

***For Lab Use Only**

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