Kligler Iron Agar

Intended Use
Kligler Iron Agar is a differential medium recommended for differentiation of members of *Enterobacteriaceae* on the basis of their ability to ferment dextrose and lactose and to produce hydrogen sulphide.

Summary
Kligler Iron Agar is a combination of Kligler's lead acetate medium and Russel's double sugar tubed medium. This medium is used for the isolation of typhoid bacilli from urine and faeces. Kligler Iron Agar differentiates lactose fermenters from the non-fermenters.

Principle
Proteose Peptone and peptone provide essential growth nutrients. Yeast extract is a source of B group vitamins while sodium chloride maintains the osmotic balance. Lactose and dextrose enable the differentiation of enteric bacilli due to change in colour of the phenol red pH indicator in response to the acid produced during the fermentation of these sugars. Ferrous sulphate and sodium thiosulphate enable the detection of hydrogen sulphide production. Fermentation of dextrose is indicated by yellow butt and that of lactose by yellow slant.
Non-lactose fermenters (*Salmonella and Shigella*) initially produce acid (yellow slant) as a result of dextrose fermentation. The concentration of dextrose being very small is rapidly exhausted. Once the dextrose is depleted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt where an acidic environment is maintained. Lactose fermenting organisms produce yellow slants and butts. Organisms incapable of fermenting either of the carbohydrates produce red slants and butts. H_2S production results in the blackening of the medium, either throughout the butt or in a ring formation near the top of the butt. Gas production is demonstrated by the presence of bubbles or cracks in the medium.

Formula*

Ingredients	**g/L**
Peptone	15.0
Yeast Extract	3.0
Cara Beef Extract#	3.0
Proteose Peptone	5.0
Sodium Chloride	5.0
Lactose	10.0
Dextrose	1.0
Sodium Thiosulphate	0.3
Ferrous Sulphate	0.2
Phenol Red	0.024
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.
#Equivalent to Beef Extract

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples - Urine, Faeces;
Food and Dairy samples;
Water samples

Specimen Collection and Handling

Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 57.52 g of the powder in 1000 mL purified / distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Mix well and distribute into tubes.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Allow to set as slopes with 1-inch butts.
6. Best reactions are obtained on freshly prepared medium.

Quality Control

Dehydrated Appearance: Light pink coloured, homogenous, free flowing powder.
Prepared Appearance: Red to reddish brown coloured, slightly opalescent gel forms in tubes as slants with 1-inch butts.
Cultural Response: Cultural characteristics observed after an incubation of 18-48 hours at 30ºC-35ºC.

Organism (ATCC)	**Growth**	**Slant**	**Butt**	**Gas**	**H_2S**
Escherichia coli (25922)	Good	A	A	+	-
Shigella flexneri serotype 2b (12022)	Good	K	A	-	-
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	K	A	+	+
Pseudomonas aeruginosa Strain Boston 41501 (27853)	Good	K	K	-	-
Citrobacter freundii (8090)	Good	A	A	+	+
Klebsiella aerogenes (13048)	Good	A	A	+	-
Proteus hauseri (13315)	Good	K	A	-	+
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	A	A	+	-

Note:
A = Acidic (Colour of the media changed to yellow)
K = Alkaline (No change in colour of the media or the colour of the media changed to pink)
+ = Positive (For H_2S positive colour of the media changed to black and for gas positive bubbles/gap/disruption observed into the media)
- = No reaction (No change in colour of the media)

Interpretation of results

1. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only.
2. An acid slant-acid butt (yellow/yellow) indicates fermentation of both dextrose and lactose.
3. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented.
4. Cracks, splits or bubbles in the medium indicate gas production.
5. A black precipitate in the butt indicates H_2S production.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. It is essential that Kligler Iron Agar be examined and reported after 18-24 hours. Early or late readings may give false results.
2. Kligler Iron Agar will grow both oxidative and fermentative organisms and care should be taken to distinguish between the two groups.
3. It is essential that air is freely available for growth on the slant and therefore do not use screw cap bottles or tubes for testing.
4. Several colonies from each primary plate should be studied separately, since mixed infections may occur.
5. It is important to stab the butt of the medium but the integrity of the medium must be maintained while stabbing. Use a straight wire for inoculation to avoid splitting the agar.
6. An organism that produces H_2S may mask acid production in the butt of the medium. However, H_2S production requires an acid environment, thus the butt portion should be considered acidic if H_2S is produced.
7. Certain species of organisms may give delayed reactions or completely fail to ferment the carbohydrate. However, in most cases, if the organism fails to ferment dextrose within 48 hours and growth is definitely present, the organism, most likely does not belong to the family *Enterobacteriaceae.*

Warranty

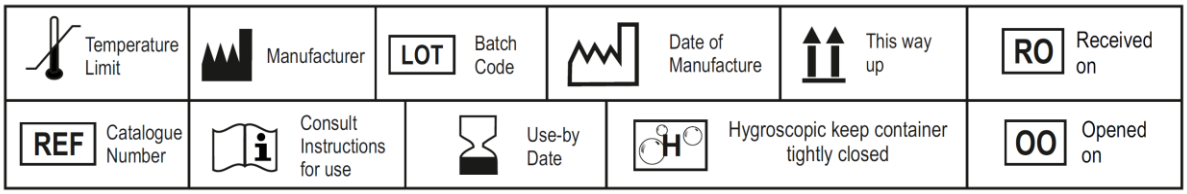
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Russel F.F; 1911, J. Med. Res.
2. Kligler, 1917, Am. J. Public Health.
3. Downes and Ito (ed.) 2001, Compendium of Methods For The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
4. Bailey and Lacy. 1927. J. Bacteriol.
5. Ewing. 1986. Edwards and Ewing's identification of the Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc. New York, N.Y.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201110070100	Dehydrated Culture Media	100 g
201110070500	Dehydrated Culture Media	500 g



Revision: 0825/VER-03

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.