ARENA
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Kligler Iron Agar

Designed for differential identification of gram-negative enteric bacillis on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

REF: BS.1/KI01.100.0100	100 Gram	REF: BS.1/KI01.250.0250	250 Gram
REF: BS.1/KI01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Kligler Iron Agar is a differential culture medium developed by combining the lead acetate medium introduced by Kligler (1,2) with Russell's Double Sugar Agar formulation (3). It is widely used for the differentiation of Salmonella, Shigella, and other enteric pathogens, including typhoid and dysentery bacilli (4). Bailey and Lacey later replaced the Andrade indicator with phenol red as the pH indicator, improving the interpretation of carbohydrate fermentation reactions (4). The medium is designed to differentiate lactose-fermenting organisms from non-lactose fermenters. It is also useful for distinguishing Salmonella Typhi from other Salmonella species, as well as differentiating Salmonella Paratyphi A from Salmonella Scottmuelleri and Salmonella Enteritidis (5).Fermentation of dextrose produces acid, causing the phenol red indicator to change from red to yellow. Because dextrose is present in a limited concentration, the acid formed is rapidly exhausted, allowing oxidation of the aerobic slant and restoration of the red color. In contrast, lactose fermentation generates a larger quantity of acid, preventing reoxidation of the indicator and resulting in a yellow coloration throughout both the slant and the butt of the medium.

METHOD PRINCIPLE

Kligler Iron Agar, in addition to Peptone, HM peptone B 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 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. 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.

MEDIA COMPOSITION

Item	Formula per liter of medium
Peptone	15 gm
Beef Extract	3 gm
Yeast Extract	3 gm
Tryptone	5 gm
Lactose	10 gm
Dextrose	1 gm
Ferrous sulphate	0.2 gm
Sodium chloride	5 gm
Sodium thiosulphate	0.3 gm
Phenol red	0.024 gm
Agar	15 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Kligler Iron Agar material safety data sheet.

STORAGE AND STABILITY

BioScien Kligler Iron Agar should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 7.4±0.2 at 25°C

PREPARATION

Suspend 57.52 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into sterile test 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 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Deterioration

The color of ***BioScien*** Kligler Iron Agar medium is Light yellow to pink homogeneous free flowing powder.Prepared Media is reddish orange in color. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Clinical samples, water samples, food and dairy samples.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile test tubes
- Incubator
- Autoclave

Performance Characteristics

Cultural characteristics observed after incubation at 35 - 37°C for 18 - 48 hours.

Organism	Slope of slant	Butt of slant	Gas	H_2S
Shigella sonnei NCTC 8574	Red	Yellow	-	-
Shigella flexneri ATCC 12022	Red	Yellow	-	-
Salmonella typhi ATCC 6539	Red	Yellow	-	+
Salmonella species	Red	Yellow	+	+
Enterobacter species	Red	Yellow	+	-
Klebsiella species	Yellow	Yellow	+	-
Escherichia coli ATCC 25922	Yellow	Yellow	+	-
Proteus mirabilis ATCC 12453	Red	Yellow	-	+
Citrobacter freundii ATCC 8090	Yellow	Yellow	+	+
Salmonella Schottmuelleri ATCC 10719	Red	Yellow	+	+
Salmonella Paratyphi A ATCC 9150	Red	Yellow	+	-
Salmonella Enteritidis ATCC 13076	Red	Yellow	+	+

+ = Positive result, - = Negative result

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

1.Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.

2. Kligler I. J., 1918, J. Exp. Med., 28:319.

3.Russell F. F., 1911, J. Med. Res., 25:217.

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.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		