ARENA BioScien

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Kligler Iron Agar

Solid medium used for the differentiation between microorganisms on the basis of dextrose and lactose fermentation and hydrogen sulfide 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 differentiates between lactose-fermenting and nonlactose-fermenting Gram-negative bacilli. It differentiates *Salmonella Typhi* from other *Salmonellae* and also *Salmonella Paratyphi A* from *Salmonella Scottmueller*i *and Salmonella Enteritidis*. Pure cultures of suspected organisms from plating media such as MacConkey Agar, Bismuth Sulphite Agar, or Deoxycholate Citrate Agar, Salmonella Shigella Agar etc. are inoculated on Kligler Iron Agar for identification.

METHOD PRINCIPLE

Phenol red is the pH indicator, which exhibits a color change in response to acid produced during the fermentation of sugars. 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. 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 glucose (dextrose). When glucose (dextrose) supply runs out 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 color of the medium indicate 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

Deterioration

The color of ***BioScien*** Kligler Iron Agar medium is cream to yellow 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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
4. 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.
5. MacFaddin, J. F. Media for isolation-cultivation-identification-maintenance of medial bacteria, Williams & Wilkins, Baltimore, MD
6. Bailey Sadie F. and Lacey G. R. (1927) *J. Bact. 13*. 182-189.

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		

EC REP

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CE