Also known as

KIA; Iron Agar; Kligler's Iron Agar

Specification

Solid differential medium for primary identification of enterobacteria based on the fermentation of two sugars and the hydrogen sulfide production according to ISO standard.

Formula * in g/L

Meat extract	3,00
Yeast extract	3,00
Peptone	20,00
Lactose	10,00
Sodium chloride	5,00
Dextrose	1,00
Ammonium ferrous citrate	0,50
Sodium tiosulfate	0,50
Phenol red	0,03
Agar	15,00

Final pH 7,4 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 58 g of powder to 1 L of distilled water and bring to the boil. Distribute in tubes and sterilize in the autoclave at 121ºC for 15 minutes. Let it solidify with a short slant and large butt.

Description

Kligler Agar is a differential medium that has all the characteristics of the 2-Sugar Russell Agar and Lead Acetate Medium for H_2S detection. In this medium, lactose fermentation and hydrogen sulfide production can be detected, allowing a presumptive identification of most enterobacteria. Sugar fermentation is shown by acid production, which turns the indicator from red to yellow. Since there is only a small amount of sugar (dextrose) in the medium, acid production due to its fermentation is very limited and re-oxidation of the indicator occurs on the surface of the medium, causing the indicator to remain red. When lactose is fermented, a large amount of acid is produced re-oxidation does not occur and the entire medium turns yellow.
Hydrogen sulfide production is indicated by the medium turning black, due to the reaction of H_2S (liberated from thiosulfate) with the iron ions presents in the ammonium iron citrate.

Technique

Kligler Iron Agar is used in slanted tubes with short slant and a generous butt, which are inoculated on the surface and also stab inoculated. The inoculum must be copious; it has to come from a solid medium, otherwise, readings may be delayed (up to additional 2-3 days). Normal incubation is 18-24 hours at 36ºC ±0,2.
Tubes with caps that allow ventilation, are recommended, such as cotton caps, cellulose caps or cap-o-test.
Should screw caps be used, do not tighten them otherwise they can hinder the re-oxidation of the indicator.
Kligler's medium provides excellent results if used freshly prepared, however if it has been prepared a few days beforehand, it is advisable to re-melt it and solidify it again to obtain more accurate readings.
A large production of H2S may make the readings difficult, and hence early readings are strongly recommended.More precise readings are obtained if Three Sugar Iron Agar is used, since this contains sucrose allowing a greater differentiation between members of Proteus, Salmonella and Shigella spp.

Quality control

Incubation temperature: 36°C ±2,0 **Incubation time:** 18-24 h
Inoculum: Stab the butt and streak the slant. Specificity according to ISO 11133.

Microorganism	Growth	Remarks
Proteus vulgaris ATCC® 6380	Good	Slant: N, blackness; Butt: Ac H2S (+)
Shigella flexneri ATCC® 12022	Good	Slant: N; Butt: Ac; H2S (-). Yellow medium
Escherichia coli ATCC® 8739	Good	Slant: Ac; Butt: Ac / Gas; H2S (-)
Escherichia coli ATCC® 25922	Good	Slant: Ac; Butt: Ac / Gas; H2S (-)
Salmonella abony NCTC® 6017	Good	Slant: N blackness; Butt: Ac H2S (+)
Salmonella typhimurium ATCC® 14028	Good	Slant: N blackness; Butt: Ac H2S (+)

References

· ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Ratón. Fla. USA.
· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th ed. APHA. Washington. DC. USA.
· ISO 6340:1995 Standard. Water Quality - Detection of Salmonella species. Geneva.
. ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· KLIGLER (1918) Modification of culture media used in the isolation and differentiation of thyphoid, dyesentery and allied bacilli. J.Exper Med. 28:319-332.
· KLIGLER (1917) A simple medium for the differentiation of members of thyphoid-parathyphoid groups. Am. J. Pub. Hlth 7:1042-1044.
· MacFADDIN, J.F. (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. William & Wilkins. Baltimore. MD. USA.
· RUSELL, 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. 25:217-220.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).