

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	Ammonium ferrous citrate.....	0.50
Yeast extract.....	3.00	Sodium tiosulfate.....	0.50
Peptone.....	20.00	Phenol red.....	0.03
Lactose.....	10.00	Agar.....	15.00
Sodium chloride.....	5.00		
Dextrose.....	1.00		

Final pH 7.4 ±0.2 at 25 °C

* 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 121°C 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 H₂S 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 H₂S (liberated from thiosulfate) with the iron ions presents in the ammonium iron citrate.

Technique

Kligler fer 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 à 36 °C ± 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 fer Agar is used, since this contains sucrose allowing a greater differentiation between members of *Proteus*, *Salmonella* and *Shigella* spp.

Quality control
Incubation temperature: 37°C ±1

Incubation time: 24 ± 3h

Inoculum: Stab the butt and streak the slant. ≥ 10³ CFU (specificity) according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020.

Microorganism
Growth
Remarks

<i>Proteus mirabilis</i> ATCC® 43071	Good	Slant:K; Butt:A; G(-); H2S (+)
<i>Shigella flexneri</i> ATCC® 12022	Good	Slant:K; Butt:A; G(-); H2S (-)
<i>Shigella sonnei</i> ATCC® 9290	Good	Slant:K; Butt:A; G(-); H2S (-)
<i>Escherichia coli</i> ATCC® 25922	Good	Slant:A; Butt:A; G(+); H2S (-)
<i>Salmonella enteritidis</i> ATCC® 13076	Good	Slant:K; Butt:A; G(-); H2S (+)
<i>Salmonella typhimurium</i> ATCC® 14028	Good	Slant:K; Butt:A; G(-); H2S (+)
<i>P. aeruginosa</i> ATCC® 27853	Good	Slant:K; Butt:K; G(-); 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 11133:2014/ Adm 1:2018. 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 typhoid, dysentery and allied bacilli. J. Exper Med. 28:319-332.
- KLIGLER (1917) A simple medium for the differentiation of members of typhoid-paratyphoid 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 °C to 30 °C).