Kligler Iron Agar

Cat. 1042

For the differentiation of Gram negative Enterobacteria

Practical information

Aplications	Categories
Differentiation	Enterobacteria

Industry: Clinical

CE
IVD

Principles and uses

Kligler Iron Agar may be used to differentiate Gram-negative Enterobacteria on the basis of carbohydrate fermentation and H2S production.

The Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose and Lactose are the fermentable carbohydrates, producing acid indicated by the Phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide, which reacts with the iron salt to give the black iron sulfide. Sodium sulfide and Ferric ammonium citrate are H2S indicators. Bacteriological agar is the solidifying agent.

Lactose non fermenters (e.g.,Salmonella and Shigella) initially produce a yellow slant due to acid formation caused by the fermentation of dextrose. Once the dextrose supply runs out 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, which remains acid (yellow butt). Lactose fermenters produce yellow slants and butts due to the fact that sufficient acid is produced in the slant to maintain an acid pH under aerobic conditions. Organisms incapable of fermenting the carbohydrates produce red slants and butts.

For best results, Kligler Iron Agar should be used on the day of preparation or melted and solidified before use.

Formula in g/L

Bacteriological agar	15	Dextrose	1
Ferric ammonium citrate	0,5	Lactose	10
Peptone mixture	20	Phenol red	0,025
Sodium chloride	5	Sodium thiosulfate	0,5

Preparation

Suspend 52 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 ºC for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1,5 - 2 cm depth.

Instructions for use

For clinical diagnosis, the type of sample is bacteria isolated from feces.
- Inoculate the tubes with the sample by stabbing the butt and streaking the surface of the tube.
- Incubate the tubes at 35±2 °C for 18-24 hours.
- Reading and interpretation of the results.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Slightly opalescent may present a slight precipita	Fine powder	Beige-pink	Pink-orange	7,4±0,2

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Microbiological test

Incubation conditions: (35±2 ºC / 24 h).

Microrganisms	Specification	Characteristic reaction
Shigella flexneri ATCC 12022	Good growth	Red slant, Yellow base, H2S (-), Gas (-)
Salmonella enteritidis ATCC 13076	Good growth	Red slant, Yellow base, H2S (+), Gas (+)
Escherichia coli ATCC 25922	Good growth	Yellow slant, Yellow base, H2S (-), Gas (+)
Proteus vulgaris ATCC 6380	Good growth	Red slant, Yellow base, H2S (+), Gas (-)
Citrobacter freundii ATCC 8090	Good growth	Yellow slant, Yellow base, H2S (+), Gas (+)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

J. Bact. 13:1 83. 1927. J. Bact. Clin. Med. 25:649, 1940.

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