Kliger Iron Agar, Modified

M078A

Intended Use:

Recommended for identification of *Yersinia enterocolitica.* Also used for differential identification of gram-negative enteric bacilli on the basis of the fermentation of dextrose, lactose and hydrogen sulphide production.

Composition**

Ingredients	g / L
HM peptone B #	3.000
Yeast extract	3.000
Tryptone	20.000
Sodium chloride	5.000
Lactose	10.000
Dextrose (Glucose)	1.000
Ferrous sulphate	0.200
Sodium thiosulphate pentahydrate	0.300
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 57.41 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in a slanted position to form slope of about one inch butts. Best reactions are obtained on freshly prepared media.

Note: Avoid overheating otherwise may produce precipitate in the medium.

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1) and Russels Double Sugar Agar (2) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (3). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other *Salmonella* and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (5). Kligler Iron Agar, Modified is used for the identification of *Yersinia enterocolitica*, as recommended by ISO Committee (6). 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.

Kligler Iron Agar, in addition to tryptone, 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 or 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.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or

Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

Type of specimen

Isolated Microorganism from clinical, food, dairy and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8)
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,11)
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(12)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Results should be noted after 18-24 hours. Else it might result in erroneous results.
2.Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 5.74% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Organism	Growth	Slant	Butt	Gas	H_2S
Escherichia coli ATCC 25922 (00013*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
Enterobacter cloacae ATCC 13047 (00083*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction,no blackening of medium
Klebsiella pneumoniae ATCC 13883 (00097*)	luxuriant	alkaline reaction, red colour of the medium	Acidic reaction, yellowing of the medium	positive reaction	negative reaction no blackening of medium
Proteus vulgaris ATCC 6380	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	Negative reaction	Positive reaction,blackening of medium

Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative reaction,no blackening of medium
Salmonella Typhi *ATCC 6539*	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive reaction,blackening of medium
Salmonella Enteritidis *ATCC 13076* (00030*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction,blackening of medium
Shigella flexneri ATCC 12022 (00126*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative reaction,no blackening of medium
Yersinia enterocolitica ATCC 27729	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	variable reaction	negative reaction,no blackening of medium
# Klebsiella aerogenes ATCC 13048 (00175*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction, no blackening of medium
Citrobacter freundii ATCC 8090	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction, blackening of medium
Salmonella Paratyphi A *ATCC 9150*	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction,no blackening of medium
Salmonella Schottmuelleri *ATCC 10719*	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive reaction, blackening of medium

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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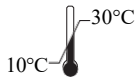
IVD ***In vitro* diagnostic medical device**



Storage temperature

EU REP

AR Experts BV
Boeingavenue 209
1119 PD Schiphol-Rijk
The Netherlands

CE Marking

Do not use if package is damaged

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