



Kligler Iron Agar

M078I

Intended Use:

Recommended for identification of *Pseudomonas* species. It can be also used for differential identification of Gram-negative enteric bacilli. The composition and performance criteria of this medium are as per the specifications laid down in ISO 13720:2010.

Composition**

ISO 13720:2010 Specification - Kligler Iron Agar

Ingredients	g / L
Beef extract	3.000
Yeast extract	3.000
Pancreatic casein peptone	20.000
Sodium chloride	5.000
Lactose	10.000
Glucose	1.000
Ammonium iron(II)sulphate hexahydrate	0.500
Sodium thiosulphate pentahydrate	0.500
Phenol red	0.025
Agar	12.000-18.000
Final pH (at 25°C)	7.4±0.2

M078I - Kligler Iron Agar

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 ammonium sulphate hexahydrate	0.500
Sodium thiosulphate pentahydrate	0.500
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 57.7 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slope with about 1 inch butts. Best reactions are obtained on freshly prepared media. Do not use screw-capped tubes or bottles.

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 (1,2) 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 is also recommended by ISO Committee for identification of *Pseudomonas* species (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 ferric ammonium 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 food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 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 5.77% 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 ₂ S
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction, no blackening of medium
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction, no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative reaction, no blackening of medium

<i>Proteus vulgaris</i> ATCC 6380	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, negative yellowing of the medium	positive reaction, blackening of medium
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, negative yellowing of the medium	negative reaction, no blackening of medium
<i>Salmonella</i> Typhi ATCC 6539	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, negative yellowing of the medium	positive reaction, blackening of medium
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, positive yellowing of the medium	positive reaction, blackening of medium
<i>Shigella flexneri</i> ATCC 12022 (00126*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, negative yellowing of the medium	negative reaction, no blackening of medium
<i>Yersinia enterocolitica</i> ATCC 27729	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, variable yellowing of the medium	negative reaction, no blackening of medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, positive yellowing of the medium	negative reaction, no blackening of medium
<i>Salmonella</i> Paratyphi A ATCC 9150	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, positive yellowing of the medium	negative reaction, no blackening of medium
<i>Citrobacter freundii</i> ATCC 8090	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, positive yellowing of the medium	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 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 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Kligler I. J., 1917, Am. J. Publ. Health, 7:104
2. Kligler I. J., 1918, J. Exp. Med., 28:319.
3. Russell F. F., 1911, J. Med. Res., 25:217.

4. Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.
5. Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc N.Y.
6. International Organization for Standardization (ISO), 2010, Draft ISO/DIS 13720.
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. .1

Revision : 04/2025

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.