KLIGLER IRON AGAR (7140)

Intended Use

Kligler Iron Agar is used for the differentiation of microorganisms on the basis of dextrose and lactose fermentation and hydrogen sulfide production.

Product Summary and Explanation

Kligler Iron Agar is a modification of Kligler's original formula, recommended to identify pure cultures of colonies picked from primary plating media.[1] The original medium was a soft nutrient agar containing dextrose, Andrade indicator and lead acetate.[1] Russell devised a medium containing glucose, lactose, and an indicator for the differentiation of lactose-fermenting and nonlactose-fermenting Gram-negative bacilli.[2] Kligler found lead acetate could detect hydrogen sulfide when combined with Russell double sugar medium for the differentiation of typhoid, partyphoid, and dysentery groups.[3] Bailey and Lacy simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. A similar medium containing sucrose, tryptone, ferrous sulfate, and thiosulfate was developed by Sulkins and Willet.[5]

Kligler Iron Agar is recommended for differentiation of enteric Gram-negative bacilli from clinical specimens and food samples.[6,7,8]

Principles of the Procedure

Kligler Iron Agar combines the principles of Russell double sugar medium and lead acetate agar into one medium. This combination permits differentiation of Gram-negative bacilli by their ability to ferment Dextrose or Lactose, and produce hydrogen sulfide. Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and vitamins required for organism growth. Ferric Ammonium Citrate and Sodium Thiosulfate are indicators of hydrogen sulfide production. Phenol Red is the pH indicator. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue	10 g
Lactose	10 g
Dextrose	1 g
Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.5 g
Phenol Red	0.025 g
Agar	15 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Distribute into test tubes and autoclave for 15 minutes at 121°C.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is reddish-orange to red, and trace to slightly hazy.

Expected Cultural Response: Cultural response in Kligler Iron Agar incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results				
		Recovery	Slant	Butt	Gas	H_2S
Escherichia coli ATCC® 25922	Heavy	Growth	A	A	+	--
Proteus mirabilis ATCC® 12453	Heavy	Growth	K	A	--	+
Pseudomonas aeruginosa ATCC® 27853	Heavy	Growth	K	K	--	--
Salmonella typhimurium ATCC® 14028	Heavy	Growth	K	A	+/-	+
Shigella flexneri ATCC® 12022	Heavy	Growth	K	A	--	--

The organisms listed are the minimum that should be used for quality control testing.
KEY: A, acid, K, alkaline, +, positive, -, negative, +/-, usually positive

Test Procedure
Stab the center of the medium into tube butt. Withdraw the needle, and streak surface of the slant. Loosen caps to allow a free exchange of air before incubating at 35°C for 18 – 38 hours. Read tubes for acid production on slant/butt, gas production, and hydrogen sulfide production.

Results
An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermenter). Cracks, splits, or bubbles in the medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production.

Storage
Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. Hydrogen sulfide producing organisms may produce a black precipitate to such a degree that the reaction in the butt is completely masked. If hydrogen sulfide is produced, dextrose is fermented even if it is not observed.[9]
2. Further biochemical tests and serological typing must be performed for definite identification and confirmation.
3. Pure cultures are essential when inoculating Kligler Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
4. Hydrogen sulfide determinations using Kligler Iron Agar should be limited to members of *Enterobacteriaceae*.[9]

Packaging
Kligler Iron Agar	**Code No.**	**7140A**	**500 g**
		7140B	**2 kg**
		7140C	**10 kg**

References

1. **Kligler, I. J.** 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health **7**:1042-1044.
2. **Russell, 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.
3. **Kligler, I. J.** 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. **28**:319-322.
4. **Bailey, S. F., and L. R. Lacy.** 1927. A modification of the Kligler lead acetate medium. J. Bacteriol. **13**:183.
5. **Sulkin, S. E., and J. C. Willett.** 1940. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J. Lab. Clin. Med. **25**:649-653.
6. **Isenberg, H. D. (ed.).** Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
8. **www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.**
9. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International, Gaithersburg, M.D.
10. **MacFaddin, J. F.** Media for isolation-cultivation-identification-maintenance of medial bacteria, Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)7372-9200 or fax us at (517)372-2006.

NEOGEN CORPORATION
620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/783-3212 • fax: 800/875-8563
neogen-info@neogen.com • www.neogen.com