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TECHNICAL PRODUCT INFORMATION

Kligler Iron Agar

Catalog No.:
T1478 16 X 125 mm Tube
T1479 16 x 125 mm Slip Cap

INTENDED USE:

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

HISTORY/SUMMARY:

In 1911, Russell[1] described a combination of two sugars in an agar medium to differentiate gram negative intestinal microorganisms. Kligler[2, 3] added lead acetate to Russell's medium and reported successful differentiation of typhoid-paratyphoid group. Kligler found lead acetate could detect hydrogen sulfide when combined with Russell double sugar medium for the differentiation of typhoid, paratyphoid, and dysentery groups.[3] Russell devised a medium containing glucose, lactose, and an indicator for the differentiation of lactose-fermenting and nonlactose-fermenting Gram-negative bacilli.[2] Bailey and Lacy simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. Kligler Iron Agar is a modification of Kligler's original formula, combining features of the Kligler's lead acetate medium with the features of Russell's double sugar agar.[4, 5] Kligler Iron Agar is recommended for differentiation of enteric Gram-negative bacilli from clinical specimens and food samples.[5]

PRINCIPLES:

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, which produces color changes of the pH indicator in response to acid production during fermentation of the sugars. Dextrose concentration is 10% of the Lactose concentration. 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.

Sodium Thiosulfate is reduced to hydrogen sulfide, which reacts with an iron salt producing the black iron sulfide. Ferric Ammonium Citrate is the hydrogen sulfide (H_2S) indicator; this reaction is mainly in the butt of the tube. The butt may be black throughout or have a ring formation near the top of the butt. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.[4, 5]

Lactose nonfermenters such as *Salmonella* and *Shigella* initially produce an acid (yellow) slant due to acid produced by fermentation of the dextrose. When the dextrose is completely fermented in the aerobic atmosphere of the slant, the reaction will revert to an alkaline (red) slant due to oxidation of the acids. The reversion does not occur in the anaerobic atmosphere of the butt, which remains acid (yellow butt). Lactose fermenters produce acid (yellow) slants and butts since enough acid is produced in the slant to maintain an acid pH under an aerobic atmosphere. Organisms which cannot ferment dextrose or lactose produce alkaline (red) slants and butts.[4, 5]

Hydrogen sulfide may be displayed as a black butt or black ring near the butt top. Gas production is shown as bubbles in the agar or splitting or displacement of the agar.[4, 5]

FORMULA:

Ingredients per liter of purified water:	
Enzymatic Digest of Casein	10.0 g
Enzymatic Digest of Animal Tissue	10.0 g
Lactose	10.0 g
Dextrose	1.0 g
Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5.0 g
Sodium Thiosulfate	0.5 g
Phenol Red	0.025 g
Agar	15.0 g

Final pH: 7.4 ± 0.2 @ 25°C

PRECAUTIONS:
This medium is for In Vitro Diagnostic Use. It supports the growth of pathogens and should be handled with caution by adequately trained personnel under the supervision of a microbiologist. Media showing signs of deterioration or contamination must not be used. Media must be brought to room temperature before use.

STORAGE:
Store media at 2-8°C, do not use beyond the expiration date.

SPECIMEN COLLECTION:
Triple Sugar Iron Agar must not be used as a medium for primary isolation of microorganisms from clinical specimens. Prior to its use inoculation of specimens on adequate media is necessary. Standard procedure for inoculation of clinical specimens must be followed.

PROCEDURE:
1. Using a sterile straight inoculating needle, select an isolated colony from the culture plate.
2. Remove tube cap, stab needle into the butt of the medium.
3. Withdraw inoculating needle to the slant and streak back and forth up the slant surface.
4. Replace cap loosely on the tube.
5. Incubate aerobically overnight (18–24 hours) at 35 ± 2°C, observe and record reactions.
6. Good growth must occur in the butt and slant, or equivocal reactions will result.

NOTE:
1. On occasion, equivocal reactions may result and may be due to the selection of mixed organisms. Whenever there is a question, the purity of the culture should be confirmed by repeat testing with a pure isolate of the organism.
2. It is recommended to streak only half way up the slant to avoid reversion of sugar to an alkaline reaction (pink/red) in the thin tip of the slant.

Some of the typical reactions of Enterobacteriaceae on Kligler's Iron Agar[4] are described as follows:

Organisms	**Slant**	**Butt**	**H_2S**	**Gas**
Escherichia coli	A	A	-	+
Shigella	K	A	-	-
Salmonella sp.	K	A	+	+
Klebsiella	A	A	-	+
Enterobacter	A	A	-	+
Proteus vulgaris	A (K)	A	+	+
Proteus mirabilis	K (A)	A	+	+
Providencia	K	A	-	±
Citrobacter	K	A	±	+
Edwardsiella	K	A	+	+
Morganella	A	A	-	±

KEY:

A = Acid
K = Alkaline
\+ = Positive
\- = Negative
+/- = usually negative, positive, growth

QUALITY CONTROL:

It is recommended the user confirm the performance characteristics of this medium. Careful selection of organisms must be made to assure maximum testing success. Incubation temperatures and other environmental conditions must be controlled to further warrant effective test results.

LIMITATIONS:

Although Kligler's Iron Agar provides valuable information in the presumptive identification of *Enterobacteriaceae*, a number of other tests must be performed before complete identification of some species is accomplished. If *Salmonella* or *Shigella* are isolated, serologic testing must be done in conjunction with biochemical testing.[4, 5]

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. Hydrogen sulfide determinations using Kligler Iron Agar should be limited to members of *Enterobacteriaceae*.[4, 5]

A number of mutant species might deviate from the typical reactions described above, the use of a wide variety of carbohydrates and other tests then become necessary. The biochemical reactions must be considered as a whole when trying to identify less common strains.[4, 5]

PERFORMANCE CHARACTERISTICS:

NEL approval for each lot of Kligler's Iron Agar is based on results obtained on a number of tests, among them bacteriological performance with challenge organisms.

REFERENCES:

1. Russell, Journal of Medical Research. 25:217, 1911
2. Kligler, Journal of Experimental Medicine, 28:319, 1918
3. Kligler, American Journal of Public Health, 7:1042, 1917
4. Difco & BBL Manual, Manual of Microbiological Media, 2003, Pages 282-284
5. Acumedia Product Information Sheet, PI7140, Rev 4, March 2011

16JAN2016