KLIGLER IRON AGAR (KIA)

INTENDED USE

Remel Kligler Iron Agar (KIA) is a solid medium recommended for use in qualitative procedures for differentiation of enteric gram-negative bacilli on the basis of dextrose and lactose fermentation and hydrogen sulfide (H_2S) production.

SUMMARY AND EXPLANATION

In 1918, Kligler described a medium for detection of H_2S and differentiation of *Salmonella* spp.[1] Bailey and Lacey further modified the medium by substituting phenol red indicator for Andrade indicator.[2] This medium became known as KIA. It is recommended by Edwards and Ewing for determination of H_2S production by enteric gram-negative bacilli.[3] Gilardi has also recommended KIA for detection of H_2S produced by some strains of *Pseudomonas*.[4]

PRINCIPLE

Casein and meat peptones supply nitrogenous compounds, amino acids, and vitamins necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Lactose and dextrose are carbohydrate sources. Phenol red is an indicator of carbohydrate fermentation. Fermentation reactions are read on the slant and in the butt, indicated by a color change from red (alkaline) to yellow (acid). The dextrose concentration in KIA is one-tenth the concentration of lactose. This serves to distinguish dextrose-only fermenting organisms from those which also ferment lactose. The small amount of acid produced in the slant during dextrose fermentation oxidizes rapidly, causing the slant to revert to alkaline (red). The yellow acid reaction is maintained in the butt due to the absence of oxygen. Lactose fermenters result in yellow slants and butts because enough acid is produced in the slant by fermentation of both sugars to maintain an acid pH under aerobic conditions. If the organism does not ferment dextrose, the slant and butt remain neutral (red). Ferric ammonium citrate is an indicator of H_2S production. If H_2S is produced from sodium thiosulfate, it reacts with ferric ammonium citrate to form a black precipitate (ferrous sulfate) in the medium. Gas production is indicated by bubbles, a splitting of the medium, or displacement of the medium.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	10.0	g	Sodium Thiosulfate	0.3	g
Lactose	10.0	g	Ferric Ammonium Citrate	0.2	g
Meat Peptone	10.0	g	Phenol Red	25.0	mg
Sodium Chloride	5.0	g	Agar	12.5	g
Dextrose	1.0	g	Demineralized Water	1000.0	ml

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. The performance of this medium is dependent on proper inoculation.
2. Using an inoculating needle, inoculate KIA with a light inoculum from a pure, 18-24 hour culture growing on solid medium. Streak the slant back and forth across the entire surface and stab to the bottom of the agar butt.
3. Incubate tube in ambient air at 33-37°C for 18-24 hours and with cap loosened.
4. Examine for fermentation reactions, gas production, and H_2S production.

INTERPRETATION OF THE TEST

Carbohydrate Fermentation:

Positive Test for Slant Reaction - Yellow (acid)
Negative Test for Slant Reaction - Red (alkaline)

Positive Test for Butt Reaction - Yellow (acid)
Negative Test for Butt Reaction - Red (alkaline)

KIA Color Reactions:

Red slant/ yellow butt - dextrose (+), lactose (-)
Yellow slant/ yellow butt - dextrose (+), lactose (+)
Red slant/ red butt - dextrose (-), lactose (-)

Hydrogen Sulfide Production:

Positive Test - Black color throughout medium, a black ring at the juncture of the slant and butt, or a black precipitate in the butt
Negative Test - No black color development

Gas Production:

Positive Test - Bubbles in the medium, cracking and displacement of medium, or separation of medium from side and bottom of tube
Negative Test - No bubbles and no separation or displacement of the medium

(Continued on back)

QUALITY CONTROL

All lot numbers of Kligler Iron Agar (KIA) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Yellow slant/ yellow butt, H_2S (-), Gas (+)
Pseudomonas aeruginosa ATCC® 27853	Ambient, 18-24 h @ 33-37°C	Red slant/ red butt, H_2S (-), Gas (-)
Salmonella enterica serovar Typhimurium ATCC® 14028	Ambient, 18-24 h @ 33-37°C	Red slant/ yellow butt, H_2S (+), Gas (+)

LIMITATIONS

1. Read and interpret KIA reactions within an 18-24 hour incubation period. A reaction read at <18 hours may be falsely interpreted as negative because the carbohydrate fermented may not yet have produced enough acid to change the phenol red indicator. A reaction read at >24 hours may be incorrect due to peptone utilization which would result in an alkaline pH shift.[5]
2. H_2S production in the butt may mask the acidity produced; however, if H_2S is present an acid condition does exist, even if it is not observable.[5]
3. This medium does not contain an inhibitor and many organism types may grow. Before inoculating KIA, be sure the organism is a catalase-positive, gram-negative bacillus.[5]
4. To enhance the alkaline condition in the slant, a free exchange of air must be permitted. If KIA tubes are tightly capped, an acid reaction caused solely by dextrose fermentation will also involve the slant. Therefore, tubes must have loosened caps during incubation.[5]
5. The H_2S indicator present in KIA is reported to be less sensitive than some methods, such as the lead acetate strip; therefore, some H_2S-positive, gram-negative bacilli may not produce H_2S in KIA.[5]
6. Before inoculation, a slight precipitate may be present on the slant. This will not effect the performance of the medium.[6]

BIBLIOGRAPHY

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4. Lennette, E.H., A. Balows, W.J. Hausler, and H.J. Shadomy. 1985. Manual of Clinical Microbiology. 4th ed. ASM, Washington, D.C.
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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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