Membrane Filter Procedure

All red or pink colonies visible with 15× magnification are counted as enterococci colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

References

1. Kenner, Clark and Kabler. 1960. Am. J. Public Health *50*:1553.
2. Kenner, Clark and Kabler. 1961. Appl. Microbiol. *9*:15.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Facklam and Moody. 1970. Appl. Microbiol. *20*:245.

Availability

Difco™ KF Streptococcus Broth

Cat. No. 212226 Dehydrated – 500 g

Difco™ TTC Solution 1%

Cat. No. 231121 Tube – 30 mL
264310 Bottle – 25 g

Kligler Iron Agar

Intended Use

Kligler Iron Agar is used for the differentiation of members of the *Enterobacteriaceae* on the basis of their ability to ferment dextrose and lactose and to liberate sulfides.

Summary and Explanation

In 1911, Russell described a new double sugar tube medium for the isolation of typhoid bacilli from urine and feces.[1] Six years later, Kligler developed a simple lead acetate medium for the differentiation of the typhoid-paratyphoid group.[2] Subsequently, Kligler evaluated culture media used in the isolation and differentiation of typhoid, dysentery and allied bacilli and endorsed Russell's medium.[3] Bailey and Lacey substituted phenol red for the Andrade indicator previously used as a pH indicator.[4]

The current formulation of Kligler Iron Agar combines features of Kligler's lead acetate medium with those of Russell's double sugar agar.

Principles of the Procedure

Kligler Iron Agar, in addition to casein and meat peptones, contains lactose and dextrose which enable the differentiation of species of enteric bacilli due to color changes of the phenol

User Quality Control

Identity Specifications

BBL™ Kligler Iron Agar

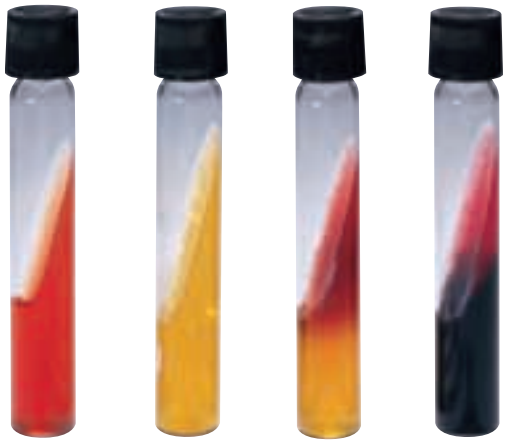
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is medium to dark, orange to red, with or without a tint of brown, clear to slightly hazy.
Prepared Appearance:	Medium to dark, orange to red, with or without a tint of brown, clear to slightly hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Kligler Iron Agar

Prepare the medium per label directions. Stab inoculate with fresh cultures and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	RECOVERY	SLANT	BUTT	H_2S
Escherichia coli	25922	Good	Acid	Acid with gas	–
Morganella morganii	8019	Good	Alkaline	Acid with or without gas	–
Pseudomonas aeruginosa	27853	Good	Alkaline	Alkaline without gas	–
Salmonella enterica subsp. *enterica* serotype Typhi	19430	Good	Alkaline	Acid without gas	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	Alkaline	Acid with gas	+
Shigella flexneri	12022	Good	Alkaline	Acid without gas	–



Uninoculated Tube | *Echerichia coli* ATCC™ 25922 | *Morganella morganii* ATCC™ 8019 | *Salmonella* Typhimurium ATCC™ 14028

Digitally signed by Bolea Petru
Date: 2019.06.07 12:15:30 EEST
Reason: MoldSign Signature
Location: Moldova

red pH indicator in response to the acid produced during the fermentation of these sugars. The dextrose concentration is only 10% of the lactose concentration. The combination of ferric ammonium citrate and sodium thiosulfate enables the detection of hydrogen sulfide production.

Lactose nonfermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of dextrose. When the dextrose supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids. The reversion does not occur in the anaerobic environment in the butt, which remains acid (yellow butt). Lactose fermenters produce yellow slants and butts because enough acid is produced in the slant to maintain an acid pH under aerobic conditions. Organisms incapable of fermenting either carbohydrate produce red slants and butts.

Hydrogen sulfide production is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar.

Formula

BBL™ Kligler Iron Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Lactose	10.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Thiosulfate	0.5	g
Agar	15.0	g
Phenol Red	25.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Cool in a slanted position such that deep butts are formed. For best results, the medium should be used on the date of preparation or melted and resolidified before use.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To inoculate, carefully touch the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant. Several colonies from each primary plate should be studied separately, since mixed infections may occur. Incubate tubes with loosened caps for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

To enhance the alkaline condition in the slant, free exchange of air must be permitted through the use of a loose closure. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

Expected Results

After incubation, record the reaction in the slant and butt, noting gas formation and hydrogen sulfide production.

Typical reactions produced by members of the *Enterobacteriaceae* (majority of the species in the particular genus) are presented in the following table.[5]

	SLANT	BUTT	GAS	H_2S
Citrobacter	Alkaline	Acid	+	+ or –
Edwardsiella	Alkaline	Acid	+	+
Escherichia coli	Acid	Acid	+	–
Enterobacter	Acid*	Acid	+	–
Morganella	Alkaline	Acid	±	–
Proteus	Alkaline or Acid	Acid	+	+
Providencia	Alkaline	Acid	±	–
Salmonella	Alkaline	Acid	+	+
Shigella	Alkaline	Acid	–	–

**May revert to alkaline even though lactose fermented (*E. aerogenes*).*

References

1. Russell. 1911. J. Med. Res. 25:217.
2. Kligler. 1917. Am. J. Public Health. 7:1041.
3. Kligler. 1918. J. Exp. Med. 28:319.
4. Bailey and Lacy. 1927. J. Bacteriol. 13:183.
5. Ewing. 1986. Edwards and Ewing's identification of the *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc. New York, N.Y.

Availability

BBL™ Kligler Iron Agar

BAM CCAM CMPH2 COMPF ISO MCM9

Cat. No.	211317	Dehydrated – 500 g
	220896	Prepared Slants – Pkg. of 10*
	220897	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*