

- Submerge a KL antitoxin strip or equivalent beneath the agar prior to solidification.
- Test samples of the finished product for performance using stable, typical control cultures.

### Procedure

Process the specimen according to accepted practices.<sup>4</sup> Inoculate the complete medium by streaking a loopful of a 24-hour culture in a single line across the plate perpendicular to (right angle to) the antitoxin strip. (Do not touch the actual strip itself.) As many as eight cultures may be tested on a single plate.<sup>5</sup> Place test isolates about 1 cm apart. Also inoculate a toxigenic (positive control) and a nontoxigenic (negative control) *C. diphtheriae* strain approximately 1 cm on either side of the test isolates.<sup>5</sup> Incubate the inverted plates under CO<sub>2</sub> at 37°C for 72 hours. Examine at 24-, 48- and 72-hour intervals.

### Expected Results

Toxigenic (virulent) cultures of *C. diphtheriae* will show fine lines of precipitation at approximately 45° angles from the culture streak. This line forms where toxin (from the bacteria) combines with antitoxin from the strip. Primary precipitin lines form an arc of identity with the precipitin line produced by an adjacent positive control strain.<sup>6</sup> Nontoxigenic strains of *C. diphtheriae* will show no lines of precipitation.

### Limitations of the Procedure

- False-positive reactions may be seen after 24 hours as weak bands near the antitoxin strip. These can be recognized when compared with the positive control.<sup>7</sup>
- Corynebacterium ulcerans* and *C. pseudotuberculosis* may also produce lines of toxin-antitoxin.<sup>8</sup>

### References

- Elek. 1948. Br. Med. J. 1:493.
- King, Frobisher and Parsons. 1949. Am. J. Public Health 39:1314.
- Hermann, Moore and Parsons. 1958. Am. J. Clin. Pathol. 29:181.
- Funke and Bernard. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
- Washington. 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York, N.Y.
- Lennette, Balows, Hausler and Truant (ed.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
- Branson. 1972. Methods in clinical bacteriology. Charles C. Thomas, Springfield, Ill.

### Availability

#### Difco™ KL Virulence Agar

Cat. No. 212192 Dehydrated – 500 g

#### Difco™ KL Virulence Enrichment

Cat. No. 298610 Tube – 12 × 20 mL\*

#### BBL™ Taxo™ KL Antitoxin Strips

Cat. No. 231740 Vial – 12 strips\*

#### BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 20 mL

\*Store at 2-8°C.

## 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.<sup>1</sup> Six years later, Kligler developed a simple lead acetate medium for the differentiation of the typhoid-paratyphoid group.<sup>2</sup> Subsequently, Kligler evaluated culture media used in the isolation and differentiation of typhoid, dysentery and allied bacilli and endorsed Russell's medium.<sup>3</sup> Bailey and Lacey substituted phenol red for the Andrade indicator previously used as a pH indicator.<sup>4</sup>

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

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.

## User Quality Control

### Identity Specifications

#### BBL™ Kligler Iron Agar

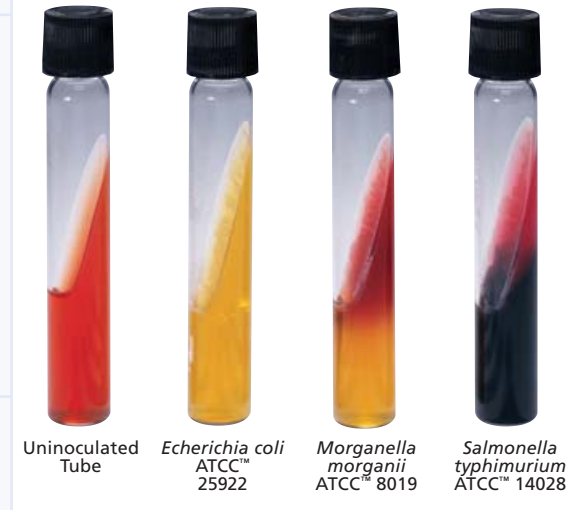
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is medium to dark, orange to red, with or without a tint of brown, clear to slightly hazy.
Prepared Appearance:	Medium to dark, orange to red, with or without 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 <sub>2</sub> S
<i>Escherichia coli</i>	25922	Good	Acid	Acid with gas	–
<i>Morganella morganii</i>	8019	Good	Alkaline	Acid with or without gas	–
<i>Pseudomonas aeruginosa</i>	27853	Good	Alkaline	Alkaline without gas	–
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	19430	Good	Alkaline	Acid without gas	+
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Good	Alkaline	Acid with gas	+
<i>Shigella flexneri</i>	12022	Good	Alkaline	Acid without gas	–



## 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

- Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense and autoclave at 121°C for 15 minutes.
- 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.
- 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.<sup>5</sup>

	SLANT	BUTT	GAS	H <sub>2</sub> S
<i>Citrobacter</i>	Alkaline	Acid	+	+ or -
<i>Edwardsiella</i>	Alkaline	Acid	+	+
<i>Escherichia coli</i>	Acid	Acid	+	-
<i>Enterobacter</i>	Acid*	Acid	+	-
<i>Morganella</i>	Alkaline	Acid	±	-
<i>Proteus</i>	Alkaline or Acid	Acid	+	+
<i>Providencia</i>	Alkaline	Acid	±	-
<i>Salmonella</i>	Alkaline	Acid	+	+
<i>Shigella</i>	Alkaline	Acid	-	-

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

## References

- Russell. 1911. *J. Med. Res.* 25:217.
- Kligler. 1917. *Am. J. Public Health.* 7:1041.
- Kligler. 1918. *J. Exp. Med.* 28:319.
- Bailey and Lacy. 1927. *J. Bacteriol.* 13:183.
- 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** **CMPH** **COMPF** **ISO** **MCM7**

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

\*Store at 2-8°C.

# Koser Citrate Medium

## Intended Use

Koser Citrate Medium is used for differentiating *Escherichia coli* from *Enterobacter aerogenes* based on citrate utilization.

## Summary and Explanation

In 1923, the work of Koser demonstrated that coli-aerogenes bacteria could be differentiated by their use of certain salts of organic acids.<sup>1</sup> Koser found that the sodium salt of citric acid (sodium citrate) is used as a source of carbon by *E. aerogenes* and not by *E. coli*. Biochemical identification schemes for identifying *E. coli* frequently include Koser citrate.

*E. coli* is an important member of the coliform group of bacteria. The coliforms are described as aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.<sup>2-5</sup> Presumptive identification is confirmed by performing biochemical tests that specifically identify *E. coli*.

## Principles of the Procedure

Koser Citrate Medium is prepared with chemically pure salts and tested to determine that no sources of carbon (other than sodium citrate) or nitrogen (other than ammonium salts) are present. Bacteria that are able to use citrate as their carbon source will grow in the medium and cause turbidity.

## Formula

### Difco™ Koser Citrate Medium

Approximate Formula* Per Liter		
Sodium Ammonium Phosphate .....	1.5	g
Monopotassium Phosphate .....	1.0	g
Magnesium Sulfate .....	0.2	g
Sodium Citrate .....	3.0	g

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

## Directions for Preparation from Dehydrated Product

- Dissolve 5.7 g of the powder in 1 L of purified water.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

- Transfer growth from a single colony or a loopful of liquid suspension and inoculate the broth medium.
- Incubate at 35 ± 2°C for 18-24 hours.

## Expected Results

Positive: ..... Turbidity  
 Negative: ..... Clear, no turbidity

## References

- Koser. 1923. *J. Bacteriol.* 8:493.
- Marshall (ed.). 1993. *Standard methods for the microbiological examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.
- U.S. Food and Drug Administration. 1995. *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, Md.
- Horwitz (ed.). 2000. *Official methods of analysis of AOAC International*, 17th ed. AOAC International, Gaithersburg, Md.
- Downes and Ito (ed.). 2001. *Compendium of methods for the microbiological examination of foods*, 4th ed. American Public Health Association, Washington, D.C.

## Availability

### Difco™ Koser Citrate Medium

**AOAC** **BAM** **COMPF** **SMD**

Cat. No. 215100 Dehydrated – 500 g

## User Quality Control

### Identity Specifications

#### Difco™ Koser Citrate Medium

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	0.57% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.57% Solution at 25°C:	pH 6.7 ± 0.2

### Cultural Response

#### Difco™ Koser Citrate Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterobacter aerogenes</i>	13048	10 <sup>3</sup>	Good
<i>Escherichia coli</i>	25922	10 <sup>3</sup>	Marked to complete inhibition