BBL™ Kligler Iron Agar Slants

L007458 • Rev. 09 • September 2014

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Kligler Iron Agar aids in the differentiation of gram-negative enteric bacilli on the basis of their ability to ferment dextrose and lactose and produce sulfides.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Using 18- to 24-h **Trypticase™** Soy Agar slant cultures, inoculate the tubes with an inoculating needle by stabbing the butt and streaking back and forth along the surface of the slant.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18–24 h for growth and reactions.
3. Expected Results

Organisms	ATCC™	Slant	Butt	Gas	H_2S
**Escherichia coli*	25922	Acid	Acid	+	–
**Salmonella enterica* subsp. *enterica* serotype Typhimurium	14028	Alkaline	Acid	+/–	+
**Shigella flexneri*	12022	Alkaline	Acid	–	–
Pseudomonas aeruginosa	27853	Alkaline	Alkaline	–	–

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV 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 produce sulfides.

V 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.

VI 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. This 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.

VII REAGENTS

Kligler Iron Agar

Approximate Formula* Per Liter Purified Water

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 .... 0.025 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[5,6] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Kligler Iron Agar Slants

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

To inoculate, carefully touch only 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 h 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.

User Quality Control: See “Quality Control Procedures.”

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be positioned in the central portion of the agar mass in solid media.

X 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):[7]

	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*).

Consult appropriate texts for additional information.[5-10]

XI LIMITATIONS OF THE PROCEDURE

Hydrogen sulfide-producing organisms may produce so much of the black precipitate, ferrous sulfide, that the acidity produced in the butt is completely masked. However, if H_2S is reduced, an acid condition does exist in the butt even if not observable and should be recorded as such.[8]

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[5-10]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Kligler Iron Agar slants are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase** Soy Agar cultures of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 14028) and *Shigella flexneri* (ATCC 12022) by streaking the slant and stabbing the butt with an inoculating needle. The tubes are incubated with loosened caps at 35 ± 2 °C and read after 18–24 h for growth and reactions. Growth of all organisms is moderate to heavy. The slant of the tube inoculated with *E. coli* shows an acid reaction while the slants of all other inoculated tubes are alkaline. *S. flexneri* produces an acid reaction in the butt, *P. aeruginosa* an alkaline reaction. *E. coli* produces acid and gas in the butt. *Salmonella* Typhimurium produces an acid reaction in the butt along with blackening of the medium; gas may or may not be present.

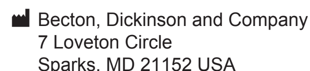
XIII AVAILABILITY

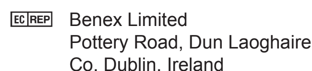
Cat. No.	Description
220896	**BBL™** Kligler Iron Agar Slants, Pkg. of 10 size K tubes
220897	**BBL™** Kligler Iron Agar Slants, Ctn. of 100 size K tubes

XIV REFERENCES

1. 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*:217-229.
2. Kligler, I.J. 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health. *7*:1042-1044.
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 N.I. Lacy. 1927. A modification of the Kligler lead acetate medium. J. Bacteriol. *13*:183-189.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
7. Ewing, W.H. 1986. Edwards and Ewing's identification of the *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc. New York.
8. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
9. Baron, E.J., L.R. Peterson, and S.M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis.
10. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

Technical Information: In the United States contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.


Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA


Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland

ATCC is a trademark of the American Type Culture Collection.
BD, BD Logo, BBL, and Trypticase are trademarks of Becton, Dickinson and Company. ©2014 BD.