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## **BIOCHEMICAL MEDIUM**

BacterTube<sup>TM</sup> Kliggler Iron Agar (KIA)

The slant agar medium is used for the identification of Enterobacteriaceae based on sugar fermentation and hydrogen sulfide (H<sub>2</sub>S) production.

Code: 08027



## BacterLab ISO 13485 ISO 9001 INSTRUCTION FOR USE



#### 1. INTENDED USE

BacterTube<sup>TM</sup> Kliggler Iron Agar (KIA) is used for the identification of enterobacteria by the rapid detection of lactose and glucose fermentation (with or without gas production), as well as the production of hydrogen sulfide.

## 2. PRINCIPLES

The fermentations of lactose and glucose, used to differentiate species of enterobacteria, result in acidification which makes phenol red (pH indicator) turn yellow. Microorganisms not fermenting lactose (Salmonella or Shigella) initially product a yellow slant due to the acidification resulting from glucose present in small quantities. When the glucose has been exhausted in the aerobic portion of the slant, the reaction becomes basic by oxidation of the acids produced, resulting in the phenomenon of a red color on the surface of the medium. This color does not appear in depth in the butt, where the color remains yellow. Bacteria fermenting lactose and glucose make the slant and the butt turn yellow because of the production of large quantities of acid. This is sufficient to maintain an acid pH on the surface. Microorganisms which ferment neither of these two sugars do not change the color of the medium. The production of H<sub>2</sub>S is revealed in the base of the medium by the appearance of black iron sulfide, due to the reduction of thiosulfate in the presence of ferric citrate. The production of gas (H<sub>2</sub>, CO<sub>2</sub>) resulting from sugar fermentations is shown by the appearance of gas bubbles or by a fragmentation of the agar.

## 3. TYPICAL COMPOSITION

For 1 liter of medium

Yeast extract	3,0 g
Meat extract	3,0 g
Glucose	1,0 g
Lactose	10,0 g
Sodium chloride	5,0 g
Sodium thiosulfate	0,5 g
Ferric ammonium citrate	0,5 g
Phenol red	25,0 mg
Aagar	15,0 g

pH of the ready-to-use medium at  $25^{\circ}C$ :  $7.4 \pm 0.2$ 

## 4. PREPARATION

The environmental tubes are ready-to-use, no preparation required.

## 5. INSTRUCTIONS FOR USE

- Allow the BacterTube<sup>TM</sup> Kliggler Iron Agar (KIA) medium tubes to reach room temperature.
- Using a suspected colony taken from a selective isolation medium, inoculate the
- butt by stabbing in the center and the inclined surface by closely spaced streaks.

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- Pure cultures taken from the center of well isolated colonies must be used to avoid cross reactions which will make identification impossible.
- Incubate at 37 °C with the caps loosely only slightly tightened in order to favor gas exchanges.

## 6. RESULTS

BacterTube<sup>TM</sup> Kliggler Iron Agar (KIA) supplies four types of information

- Glucose fermentation:
  - Red butt: glucose not fermented
  - Yellow butt: glucose fermented
- Lactose fermentation:
  - Red slope: lactose not fermented
  - Yellow slope: lactose fermented
- Gas production: Appearance of bubbles in the butt
- Formation of H2S: Production of a black color between the butt and the slope.

#### **NOTE**

- Do not read sugar fermentation characteristics before 18 hours.
- The amount of H<sub>2</sub>S produced may obscure the glucose fermentation process. If this
  occurs, it should be recorded that glucose fermentation has taken place. Check for gas
  production characteristics.
- Tests on KIA cannot differentiate all lactose-slow fermenting bacteria from fecal samples.
- Tests on KIA do not distinguish Salmonella from Citrobacter.
- Gas production testing on TSI is more effective than on KIA

## 7. QUALITY CONTROL

**BacterLab** ensures the quality of each product batch by testing with ATCC reference strains.

Reference strain	Incubation conditions	Expected result			
				Gas	
		Lactose	Glucose	production	$H_2S$
Escherichia coli ATCC 25922		+	+	+	-
Salmonella Typhimurium ATCC		-	+	+	+
14028	18 - 24 hours at				
Shigella flexneri ATCC12022	$35 - 37^{\circ}$ C, aerobic	-	+	-	-
Pseudomonas aeruginosa					
ATCC27853		-	-	-	_

## 8. STORAGE AND TRANSPORT CONDITIONS

- Storage:  $2 8^{\circ}$ C.
- Transportation: Ambient temperature.
- 9. PACKAGING



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Packaging: 50 tubes/ box or as per customer request...

#### 10. SHELF LIFE

- Expiration Date: 3 months from the manufacturing date.

## 11. BIBLIOGRAPHY

- Russel, F.F. 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. Journal of Medical Research, 25: 217-229.
- Kligler, I.J. 1917. A simple medium for the differentiation of the members of the typhoid-paratyphoid group. American Journal of Public Health, 7: 1042-1044.
- Kligler, I.J. 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. Journal of Experimental Medicine, 28: 319-322.
- Buttiaux, R. 1951. L'Analyse bactériologique des eaux de consommation. Editions médicales Flammarion, Paris.
- Buttiaux, R., Beerens, H., & Tacquet, A. 1962. Manuel de techniques bactériologiques.
   Editions médicales Flammarion, Paris.
- NF U47-100. July 2007. Animal health analysis methods. Isolation and identification of all or specific serovars of Salmonella in the environment of animal production.
- NF U47-101. November 2007. Animal health analysis methods. Isolation and identification of all or specific serovars of Salmonella in birds.
- NF U47-102. January 2008. Animal health analysis methods. Isolation and identification of all or specific serovars of Salmonella in mammals.
- Solabia Biokar Diagnostics. (2023). Kligler Iron Agar Technical Documentation.
   Retrieved from <a href="https://www.solabia.com/biokar-diagnostics/product/kligler-iron-agar/?documentation=5345&wpnonce=0212bf7863">https://www.solabia.com/biokar-diagnostics/product/kligler-iron-agar/?documentation=5345&wpnonce=0212bf7863</a>.