TECNICAL DATA SHEET

KLIGLER IRON AGAR

IDENTIFICATION OF ENTEROBACTERIA

1 INTENDED USE

Kligler Iron Agar 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 HISTORY

In 1911, Russell described a medium containing two sugars for the isolation of typhoid bacilli in urine.
Six years later, Kligler developed a nutrient medium with glucose, Andrade's indicator and lead acetate for the differentiation of bacilli from the typhi and paratyphi groups. While testing this medium with other combinations of ingredients, Kligler found that Russell's medium containing Andrade's indicator and lead acetate led to an excellent differentiation of salmonellae. Bailey and Lacey subsequently recommended using phenol red as pH indicator, replacing Andrade's indicator which was less adapted to this type of reaction.
Sulkin and Willett used sodium thiosulfate and ferrous sulfate to demonstrate hydrogen sulfide production.

3 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_2S 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_2, CO_2) resulting from sugar fermentations is shown by the appearance of gas bubbles or by a fragmentation of the agar.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 20,0 g
- 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
- Bacteriological agar..... 15,0 g

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

5 PREPARATION

- DIssolve 58,0 g of dehydrated media (BK034) into one liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into tubes.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Incline the tubes so that they obtain a butt of at least 3 cm in height and an oblique slant.

✓ **Reconstitution :**
58,0 g/L

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

- 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.
- 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.

✓ **Inoculation :**
Central stab and streaking on inclined surface

✓ **Incubation :**
24 h at 37 °C

7 RESULTS

Kligler's medium 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 H_2S :
- Production of a black color between the butt and the slope.

Typical reactions are presented in the following table :

Species	Lactose fermentation	Glucose fermentation	Gas production	Production of H_2S
Salmonella Typhi [2]	-	+	-	+
Salmonella Paratyphi *A* [2]	-	+	+	-
Salmonella Choleraesuis [2]	-	+	+	-
Salmonella Pullorum [2]	-	+	+	+
Salmonella Paratyphi *B* [2]	-	+	+	+
Salmonella Typhimurium [2]	-	+	+	+
Salmonella Enteritidis [2]	-	+	+	+
Salmonella Gallinarum [2]	-	+	-	+
Shigella dysenteriae	-	+	-	-
Shigella flexneri	-	+	-	-
Shigella sonnei	-	+	-	-
Shigella boydii	-	+	-	-
Proteus vulgaris	-	+	[+]	+
Proteus mirabilis	-	+	+	+
Proteus morganii	-	+	+	-
Proteus rettgeri	-	+	-	-
Serratia marcescens	-	+	-	-

Species	Lactose fermentation	Glucose fermentation	Gas production	Production of H_2S
Enterobacter hafniae	-	+	+	-
Enterobacter aerogenes	+	+	+	-
Enterobacter cloacae	+	+	+	-
Escherichia coli (1)	+	+	+	-
Citrobacter freundii	+	+	+	+
Klebsiella pneumoniae	+	+	+	-
Alcaligenes faecalis	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Yersinia enterocolitica	-	-	-	-

(1) Certain strains of *Escherichia coli* do not ferment lactose or do so only very late.
(2) In the case where interpretation may suggest the presence of salmonellae, it is possible to use Kligler cultures to detect β-galactosidase, urease and lysine-decarboxylase.

8 QUALITY CONTROL

Dehydrated media : pinkish powder, free-flowing and homogeneous.
Prepared media : Red-orange agar.

Typical culture response after 24 hours of incubation at 37 °C :

Microorganisms		Growth	Lactose fermentation	Glucose fermentation	H_2S production	Gas production
Escherichia coli	WDCM 00179	Bonne	+	+	-	+
Pseudomonas aeruginosa	WDCM 00026	Bonne	-	-	-	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in tubes, non-inclined (*) : 180 days at 2-8 °C.
Prepared media in tubes, inclined (*) : 8 days at 2-8 °C.
(*)Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

Note : It is recommended, when the media has not been used in the over 8 days that follows media preparation, to regenerate the tubes in a boiling water batch and to let solidify again tubes in the proper position.

10 PACKAGING

Dehydrated media :
500 g bottle BK034HA

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : KLIGLER IRON AGAR_ENv9
Creation date : 04-2001
Updated : 01-2018
Origin of revision : Bibliography.