KLIGLER IRON AGAR

Used for the differentiation of *Enterobacteriaceae*

Code: KM1040

Typical formula	**(g/l)**
Beef Extract	3.00
Yeast Extract	3.00
Peptocomplex	20.00
Lactose	10.00
Glucose	1.00
Ferrous Sulphate	0.20
Sodium Thiosulphate	0.30
Sodium Chloride	5.00
Phenol Red	0.024
Agar	12.00

pH 7.4 +/- 0.2

Directions
Suspend 54.5g in 1000ml of cold distilled water. Heat to boiling, distribute and autoclave at 121°C for 15 minutes. Cool in a slanting position to obtain deep butts and short slopes.

Description
Kligler Iron Agar is a solid medium used to distinguish between *Enterobacteriaceae* on the basis of their ability to ferment lactose and/or glucose and to produce hydrogen sulphide.
The fermentation of the sugars present can occur either aerobically (on the slope surface) or anaerobically (in the butt), with or without gas production ($CO_2 + H_2$).

Method
To perform the test, dispense 8ml of medium in test tubes and allow to solidify in a slanting position so as to produce a short slope and a butt about 3cm deep.
Inoculate by stabbing the butt and abundantly streaking the slope. After 18-24 hours of incubation at 37°C check the colour of the medium both in the butt and at the slope. Also check for the presence of gas in the butt and the presence of the black precipitate (H_2S). The table below summarises the culture characteristics of some microorganisms on Kligler Iron Agar:

Microorganisms	**Slope**	**Butt**	**H_2S**
Escherichia	A/K	A/G or A	V
Klebsiella	A	A/G	-
Enterobacter	A	A/G	-
Citrobacter	A/K	A/G	+
Salmonella paratyphi	A/K	A	-
Salmonella spp.	K	A/G	+
Salmonella arizonae	K	A/G	+
Proteus morganii	NC	A	-
Proteus vulgaris	NC	A	+
Shigella	NC	A	-

KEY
K = alkaline reaction
A = acid reaction
NC = no reaction
V= variable reaction
A/G = acid reaction with gas

Quality assurance (37°C-3 days)
E.coli ATCC 25922: yellow slope and butt, gas positive, H_2S negative
S.typhimurium ATCC 14028: red slope, yellow butt, gas and H_2S positive

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 7 days at 2-8°C

Reference
Mac Faddin, J.F. (1976) - Biochemical Tests for Identification of Medical Bacteria.

KANAMYCIN AESCULIN AZIDE AGAR BASE

A medium for the isolation of enterococci in foodstuffs

Code: KM1038

Typical formula	**(g/l)**
Tryptone	20.00
Yeast Extract	5.00
Sodium Chloride	5.00
Sodium Citrate	1.00
Aesculin	1.00
Fe-Ammonium Citrate	0.50
Sodium Azide	0.15
Agar	10.00

Supplemented with:
Kanamycin Sulphate 10 mg

pH 7.0 ± 0.2

Directions
Suspend 21.3g in 500 ml of cold distilled water. Heat to boiling with agitation, sterilise in the autoclave at 121°C for 15 minutes and cool to 50°C. Dissolve one vial of Kanamycin Selective Supplement with 5ml of sterile distilled water and add to the medium base.

Description
Kanamycin Aesculin Azide Agar is prepared according to the typical formulation described by Mossel et al. It is recommended for the detection of enterococci in foodstuffs. The medium contains kanamycin sulphate and sodium azide as inhibitory compounds and an indicator system (aesculin, ferric ammonium citrate) to detect the aesculin-hydrolysing bacteria. The enterococci will grow on the medium with grey colonies surrounded by a brown-black zone.

Method
Prepare tenfold dilutions of sample with peptone water. Within 3 hours from the sample preparation, spread 0.1ml of the inoculum onto the plates. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium). Consider the result positive for the enterococci when round, grey colonies, about 2mm in diameter surrounded by a brown-black zone are observed.

Quality assurance (37°C-24hrs)

Productivity control
E.faecium ATCC 19434: growth, grey or white colonies with black halo
E.bovis ATCC 27960: growth, grey or white colonies with black halo

Selectivity control
E.coli ATCC 25922: inhibited
B.subtilis ATCC 6633: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: 7 days at 2-8°C

Reference
Mossel, D.A.A., Bijker, P.G.H., Eelderink, I. (1978) Arch. Lebensmittel Hjg. 29, 121-127

KF STREPTOCOCUS BROTH

A selective liquid medium for the isolation and enumeration of enterococci with the MPN technique

Code: KM4042

Typical formula	**(g/l)**
Peptone mix	10.0
Yeast Extract	10.0
Sodium Chloride	5.0
Na Glycerophosphate	10.0
Maltose	20.0
Lactose	1.0
Sodium Azide	400 mg
Bromocresol Purple	15 mg

pH 7.2 +/- 0.2

Directions
Suspend 56.4g in 1000ml of cold distilled water, heat to dissolve completely, distribute 10 ml into 16x160 mm tubes and autoclave at 121°C for 10 minutes.

WARNING: Potentially hazardous as this medium contains azide.

Method
The enumeration of enterococci is carried out in tubes, according to the method of the most probable number; vary the amount of inoculum (in multiples or fractions of 1 ml) depending on the type of specimen preparing at least five tubes of each dilution. Incubate at 37°C for 24 hours, and observe if growth has occurred and a yellow colour has developed without gas production; if not, continue incubation for a further 24 hours. Calculate the result using the appropriate tables, and express it as the most probable presumptive number. If the tubes show a gas production, confirm the presumptive result with a Gram staining and with a subculture in Ethyl Violet Azide Broth.

Quality Control (37°C-24hrs)

Productivity control
E.faecalis ATCC 19433: good growth

Selectivity control
E.coli ATCC 25922: inhibited

Storage
Dehydrated media: 15-30°C
User prepared tubes: up to 7 days at 2-8°C

References
Kenner, B.A., Clark, H.F. & Kabler, P.W. (1961). AppL. Microbiol., 9,15.

KING AGAR A (Pseud. Agar P) &
KINGS AGAR B (Pseud. Agar F)

Modified King medium A to test the pyocianin production for the identification of *Pseudomonas aeruginos*a.

Modified King medium B to test the fluorescin production for the identification of *Pseudomonas aeruginos*a.

Code: (Pseud. P) - KM4022A
Code: (Pseud. F) - KM4012B

Typical formula (Pseud.Agar P)	**(g/l)**
Bacteriological Peptone	20.0
Magnesium Chloride	1.4
Potassium Sulphate	10.0
Agar	15.0

pH 7.2 ±0.2

Typical formula (Pseud.Agar F)	**(g/l)**
Bacteriological Peptone	10.0
Tryptone	10.0
Magnesium Sulphate	1.5
Dipotassium Phosphate	1.5
Agar	15.0

pH 7.2 ±0.2

Directions

Pseud. P - KM4022A

Suspend 46.4 g of powder into 1 litre distilled water with glycerol 10 ml and let it soak for 10 minutes. Heat constantly stirring until it boils. Distribute into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes. If tubes are used, let them solidify with short slant and good butt.

Pseud. F - KM4012B

Suspend 38g in 1 litre of cold distilled water and add 10ml of glycerol. Heat to boiling, distribute and sterilise by autoclaving at 121° C for 15minutes.

Description

The A medium was formulated by King, Ward and Raney in 1954 to enhance the pyocianin production on *Pseudomonas aeruginos*a. The blue pigment Pyocianin diffuses into the culture
medium and its production varies depending on the *Pseudomonas aeruginosa* strains and the growth conditions. Sometimes, although this medium enhances especially blue pigment production, it is possible that green (pioverdine) or brown (piomelanine) pigments appear and mask pyocianin. Nevertheless, fluorescence and other Pseudomonas pigments can be noticed on other more convenient media like Pseud. F Agar. Slanted tubes or Petri dishes are inoculated by superficial streaking and are then incubated at 30-32°C for 4-5 days. Petri plates usage has the inconvenience of strong medium dehydration during incubation. Therefore, it is better to use slanted tubes being

careful with the aeration, loosening the screw caps or changing them for cotton or aluminum caps. On recently isolated pathologic materials strains, pigment production is often shown early, after 24-48 hours of incubation, but if they come from water, food or soil, pigmentation can be late. When pigment has not got the usual blue colour, it is due to the production of two or more coloured substances. At this time, and if it is not confirmed on other culture medium, it is recommended to confirm by extraction: on the culture slant, 0,5-1 ml chloroform is added, and it is stirred for a few minutes until the pyocianin is diffused, that makes the dissolvent blue. After that, chloroform is acidified with a few drops of ClH, obtaining a rapid change from blue to red, fact that confirms the pyocianin presence.

References

KING E.O., M.WARD y D.E.RANEY (1954) J.Lab.Clin.Med. 44:301
CeNAN. Métodos Recomendables para el Examen Microbiológico de Alimentos y Bebidas. Madrid, 1976Spanish legislation:O.M. 8.5.1987 del M.R.C. y S.G. (B.O.E. 13.5.1987)