

TECHNICAL DATA SHEET

CLED AGAR

DETECTION AND ENUMERATION OF URINARY TRACT MICROORGANISMS

1 INTENDED USE

CLED (Cystine Lactose Electrolyte Deficient) agar is used for the isolation, enumeration and differentiation of urinary tract microorganisms.

2 HISTORY

In 1960, Sandys studied a way to avoid the invasion of plates by *Proteus*, using a media devoid of electrolytes, thereby favoring the observation of colonies formed by the other infecting microorganisms. The media was then modified by Mackey & Sandys via the incorporation of cystine in order to favor the growth of coliforms. This new formulation, applied to urinary tract infections, was used with success in the manufacturing of dipslides destined to be immersed in the urine samples.

3 PRINCIPLES

The fermentation of lactose to acid is demonstrated through the color change from green to yellow of the pH indicator, bromothymol blue.

Cystine favors the growth of coliforms that usually give rise to small colonies on the media.

The electrolyte deficiency reduces the invasion by *Proteus*.

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Pancreatic digest of gelatin	4,0 g
- Tryptone	4,0 g
- Meat extract.....	3,0 g
- L-cystine	128,0 mg
- Lactose.....	10,0 g
- Bromothymol blue	20,0 mg
- Bacteriological agar.....	15,0 g

pH of the ready-to-use media at 25 °C : 7,3 ± 0,2.

5 PREPARATION

- Suspend 36,1 g of dehydrated media (BK020) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring until complete dissolution.
- Divide into tubes or vials.
- Sterilize in an autoclave at 115 °C for 20 minutes.
- Cool and maintain at 44-47 °C.
- Pour into sterile Petri dishes and let solidify on a cold surface.

✓ **Reconstitution :**
36,1 g/L

✓ **Sterilization :**
20 min at 115 °C

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator, covers partially removed.
- Inoculate the sample.
- Incubate at 37 °C for 18 to 24 hours.

Note : A longer incubation period, greater than 24 hours may lead to the re-alkalinization of the media, which will modify the colony colors.

7 RESULTS

The colony aspects are as follows :

Characteristics	Microorganisms
Large yellow-gold colonies, surrounded by a yellow halo	<i>Escherichia coli</i> , <i>Citrobacter</i>
Large yellow-gold and viscous colonies, surrounded by a yellow halo	<i>Enterobacter</i> , <i>Klebsiella</i>
Large transparent colonies, surrounded by a blue halo	<i>Proteus</i> , <i>Serratia</i>
Large green colonies, with a brownish center, surrounded by a blue halo	<i>Pseudomonas</i>
Small, pale yellow opaque colonies	Streptococci
Very small yellow, opaque colonies	Staphylococci
Small, grey colonies	<i>Corynebacterium</i>

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : Beige powder, free-flowing and homogeneous.

Prepared media : Blue-green, limpid agar.

Typical culture response after 24 hours of incubation at 37 °C, qualitative method of inoculation

Microorganisms	Growth	Characteristics
<i>Escherichia coli</i> WDCM 00090	Good, score 2	Yellow colonies with dark center
<i>Enterobacter aerogenes</i> WDCM 00175	Good, score 2	Yellowish colonies
<i>Salmonella</i> Enteritidis WDCM 00030	Good, score 2	Blue colonies
<i>Proteus vulgaris</i> ATCC 13315	Good, score 2	Blue colonies, without invasion
<i>Staphylococcus aureus</i> WDCM 00034	Good, score 2	Dark yellow small colonies

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

The expiration date is indicated on the label.

Prepared media in vials (*) : 180 days at 2-8 °C.

Prepared media in plates (*) : 30 days at 2-8 °C.

(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK020HA

11 BIBLIOGRAPHY

Sandys, G.H. 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol., 17: 224.

Benner, E.J. 1970. Simple disposable method for quantitative cultures of urine. Appl. Microbiol., 19 (3): 409.

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.

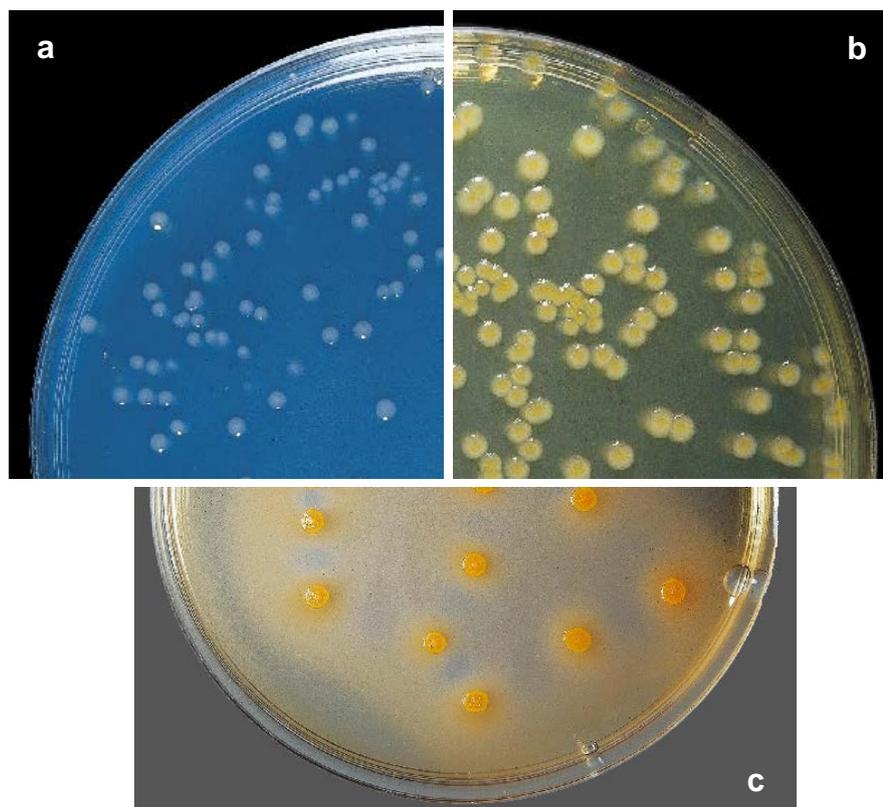
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CLED agar

Isolation, enumeration and differentiation of urinary tract microorganisms.

Results :

Growth obtained after 24 hours of incubation at 37 °C.



- (a) *Proteus* : Transparent colonies on a blue background (or surrounded by a blue halo), without invasion.
- (b) *Staphylococci* : small yellow and opaque colonies, without halo.
- (c) *Escherichia coli* : large yellow to gold colonies, surrounded by a yellow halo.