PRODUCT SPECIFICATION SHEET

C.L.E.D. (with Bromo Thymol Blue) (DM053)

Intended Use

C.L.E.D. Agar with Bromo Thymol Blue (DM053) is recommended for isolation, enumeration and identification of urinary pathogens on the basis of lactose fermentation.

Product Summary and Explanation

On a solid medium, Sandys reported that swarming of *Proteus* species can be controlled by restricting the electrolytes[1]. Formerly swarming of *Proteus* was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium[1]. Later on Sandys medium was modified by Mackey and Sandys[2], by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromo thymol blue. This formulation was further modified by the same authors, called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and by including L-cystine for promoting the growth of cystine dependant dwarf colony coliforms[3]. This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dip stick procedures and as dip inoculum transport medium for urine specimens[2, 3, 4].

Principles of the Procedure

Peptic digest of animal tissue, beef extract, casein enzymic hydrolysate provide essential growth nutrients. Lactose is the fermentable sugar. L-cystine supports the growth of dwarf coliform colony. Bromo thymol blue is the pH indicator which turns yellow at acidic pH.

Bacteriuria may be quantitated by inoculating the surface of an agar medium by proper dilution. Inoculate the medium immediately after urine collection. It can also be inoculated by calibrated loop or duplicate dilution pour plate methods.[5, 6] *Shigella* species may not grow on this medium. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than) etc. may result in low bacterial count from infected patients.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.00
Beef extract	3.00
Casein enzymic hydrolysate	4.00
Lactose	10.00
L-Cystine	0.128
Bromothymol blue	0.02
Agar	15.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT, mainly irritating to eyes, respiratory system, and skin. Handle in accordance with good laboratory hygiene and safety practice. Wash hands before breaks and at the end of workday. To protect, use safety glasses and gloves during handling.
3. Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
4. Do not let product enter drains.
5. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 36.15 g of the medium in one liter of distilled water/purified water.
2. Heat to boiling to dissolve the medium completely.

3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow, homogeneous, free flowing powder
Solution	3.61% Solution in Distilled or deionized water is soluble on boiling, Green colored and clear to slightly opalescent
Prepared Medium	Green coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 3.61% Solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.5% Agar Gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	*Enterococcus faecalis ATCC 29212*	50-100	good- luxuriant	>=70%	slight yellowish or greenish
2.	*Escherichia coli ATCC 25922*	50-100	good- luxuriant	>=70%	yellow, opaque, centre slightly deeper yellow
3.	*Klebsiella pneumoniae ATCC 13883*	50-100	good- luxuriant	>=70%	yellow to whitish blue
4.	*Proteus vulgaris ATCC 13315*	50-100	good- luxuriant	>=70%	blue
5.	*Salmonella typhi ATCC 6539*	50-100	good- luxuriant	>=70%	bluish
6.	*Staphylococcus aureus ATCC 25923*	50-100	good- luxuriant	>=70%	deep yellow

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Observe aseptic techniques.
2. Use standard procedures to obtain isolated colonies from specimens.
3. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.
4. Confirmatory tests should be further carried out for identification of isolated colonies.

Results

After 18 – 24 hours of incubation at 35 ± 2°C, Typical colonial morphology on C.L.E.D. Agar with Bromo Thymol Blue is as follows:

Enterococcus faecalis ATCC 29212 ---------- slight yellowish or greenish
Escherichia coli ATCC 25922 ---------- yellow, opaque, centre slightly deeper yellow
Klebsiella pneumoniae ATCC 13883 ---------- yellow to whitish blue
Proteus vulgaris ATCC 13315 ---------- blue
Salmonella Typhi ATCC 6539 ---------- bluish
Staphylococcus aureus ATCC 25923 ---------- deep yellow

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical and serological tests are performed for complete identification.

Packaging

Product Name : C.L.E.D. Agar with Bromo Thymol Blue
Product Code : DM053
Available Pack sizes : 100gm / 500gm

References

1. Sandys, 1960, J. Med. Lab. Technol., 17:224.
2. Mackey and Sandys, 1965, Br. Med. J., 2:1286.
3. MacKey and Sandys, 1966, Br. Med. J., 1:1173.
4. Dixson J. M. S. and Clark M. A., 1968, Conc. Med. Assoc. J., 99 (15)
5. Benner E. J., 1970, , Appl. Microbiol., 19(3), 409
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore

Further Information

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