PRODUCT SPECIFICATION SHEET

C.L.E.D. Agar w/Andrade Indicator (DM052)

Intended Use
C.L.E.D. Agar w/Andrade Indicator (DM052) is recommended for isolation and differentiation of urinary pathogens on the basis of lactose fermentation.

Product Summary and Explanation
Sandys reported a new technique where the swarming of *Proteus* on an agar medium could be prevented by restricting the electrolyte content in the culture medium.[1] Sandys Medium was modified by Mackey and Sandys,[2] by replacing mannitol with lactose and sucrose and elevating the concentration of agar and bromothymol blue. The same authors further modified this medium by retaining the lactose (deleting sucrose) and by including L-cystine for promoting the growth of cystine-dependent dwarf coliform colony.[3] This later modified medium was designated as C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) Medium. This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Agar is also recommended for dip stick procedures and as dip inoculum transport medium for urine specimens.[2,3,4]

Principles of the Procedure
C.L.E.D. Agar was further modified by Bevis[5] by incorporation of Andrades indicator. This medium provides sharper differentiation between lactose-fermenters (LF) and lactose-non-fermenters (NLF).[5] Addition of Andrade's indicator enhances the appearance of colony and aids in the identification of microorganisms.
At different pH values, the colour of the medium varies from the standard medium, which is well documented by Bevis.[5]

pH	Colour of C.L.E.D. medium
7.4	deep blue
7.0	bluish grey
6.8	pale grey
6.6	pinkish grey
6.4	bright red with whitish tinge
6.0	bright red

For better results, the medium should not be incubated for more than 24 hours because if lactose fermenters predominate, the entire medium may turn pink masking the presence of non-lactose fermenters. Inoculate the medium immediately after urine collection. *Shigella* species may not grow on this medium. Prior initiation of antibiotic therapy, low urine pH (less than 5) etc. may result in low urine 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
Andrade indicator	0.10
Agar	15.00
Final pH: 7.5 ± 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 36.25 g of the medium in one liter of 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	Light yellow to greyish yellow, homogeneous, free flowing powder
Prepared Medium	Greenish blue clear to slightly opalescent gel forms in Petri plates
Reaction of 3.62% Solution	pH 7.5 ± 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.	*Enterobacter aerogenes* ATCC 13048	50-100	good- luxuriant	>=70%	greyish green, mucoid
2.	*Enterococcus faecalis ATCC 29212*	50-100	good- luxuriant	>=70%	orange-yellow or greenish
3.	*Escherichia coli* ATCC 25922	50-100	good- luxuriant	>=70%	bright pink with pink halo
4.	*Proteus mirabilis* ATCC 25933	50-100	good- luxuriant	>=70%	blue-green
5.	*Staphylococcus aureus* ATCC 25923	50-100	good- luxuriant	>=70%	golden-yellow
6.	*Streptococcus pyogenes ATCC 19615*	50-100	good- luxuriant	>=70%	greyish green

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

Test Procedure

1. Observe aseptic techniques. Use standard procedures to obtain isolated colonies from specimens.
2. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.
3. 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. Medium w/Andrade Indicator is as follows:

Enterobacter aerogenes ATCC 13048 ---------- greyish green, mucoid
Enterococcus faecalis ATCC 29212 ---------- orange-yellow or greenish
Escherichia coli ATCC 25922 ---------- bright pink with pink halo
Proteus mirabilis ATCC 25933 ---------- blue-green
Staphylococcus aureus ATCC 25923 ---------- golden-yellow
Streptococcus pyogenes ATCC 19615 ---------- greyish green

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. Medium w/Andrade Indicator
Product Code : DM052
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. Bevis T. D., 1968, J. Med. Lab. Technol., 25:38.

Further Information
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