C.L.E.D. Agar with Andrade Indicator

Intended Use
C.L.E.D. Agar with Andrade Indicator is used for the isolation, enumeration and presumptive identification of microorganisms from urine, giving good colonial differentiation on the basis of lactose fermentation.

Summary
Sandys observed that restricting the electrolytes in a solid medium might prevent the swarming of *Proteus*. Previous chemical methods used for inhibiting the swarming of Proteus included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulphonamides to the culture medium. This electrolyte deficient medium was modified for particular use in urine culture by substituting lactose and sucrose instead of mannitol and increasing the concentrations of bromothymol blue indicator and agar. The medium was further modified by the incorporation of cystine in order to enhance the growth of cystine-dependent “dwarf colony” coliforms and by the deletion of sucrose. This new medium, Cystine-Lactose- Electrolyte-Deficient (C.L.E.D.) Agar is ideal for dip-inoculation techniques and for urinary bacteriology in general.

Principle
In C.L.E.D. Agar Medium Andrade Indicator (Acid Fuchsin in 1N Sodium Hydroxide) is incorporated. The essential growth nutrients are supplied by peptone, tryptone and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Addition of Andrade 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.

pH	Colour of the medium
7.4	deep blue
7.0	bluish grey
6.8	pale grey
6.6	pinkish grey
6.4	bright red with whitish tinge
1.0	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*
Ingredients	g/L
Lactose	10.0
Tryptone	4.0
Peptone	4.0
Beef Extract	3.0
L-Cystine	0.128
Andrade indicator	0.10
Bromothymol Blue	0.02
Agar	15.0
Final pH (at 25°C)	7.5 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical: Urine sample

Specimen Collection and Handling

Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 36.25 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Light yellow to greyish yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Greenish blue - blue coloured, clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 35°C-37°C.

Organism (ATCC)	**Growth**	**Colour of Colony**
Staphylococcus aureus subsp. *aureus* (25923)	Good	Golden yellow
Escherichia coli (25922)	Good	Faint pink with pink halo
Enterococcus faecalis (29212)	Good	Orange
Proteus mirabilis (25933)	Good	Blue
Klebsiella aerogenes (13048)	Good	Greyish green, mucoid
Streptococcus pyogenes Strain Bruno (19615)	Good	Greyish green

Interpretation of Results

1. Count the number of colonies on the plate or dipstick. Multiply by the dilution factor to convert the count to CFU per mL of the sample.
2. Contaminant bacteria usually appear in low numbers and vary in colony morphology.
3. Urinary pathogens will usually yield high counts having uniform colonial morphology and should not be sub cultured directly to routine media for identification and susceptibility testing.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. The medium should not be incubated for more than 24 hours since, if lactose fermenters predominate, the whole medium may turn pink, masking the presence of non-lactose fermenters.
2. Factors that may cause urine counts from infected patients to be low include: rapid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.
3. *Shigella* species may not grow on this medium.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Bevis, T.D. (1968). A modified electrolyte- deficient culture medium. J.Med. Lab. Tech., 25: 38-41.
2. Mackey, J.P. and Sandys, G.H. (1966). Diagnosis of urinary infections, Brit. Med. J., 1: 1173.
3. Sandys, G.H. (1960). A new medium for preventing swarming of Proteus spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Tech., 17: 224-233.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201030090100	Dehydrate Culture Media	100 g
201030090500	Dehydrate Culture Media	500 g
201030092500	Dehydrate Culture Media	2.5 k
203030270100	Bottle Media	100 mL