CYSTINE LACTOSE ELECTROLYTE DEFICIENT (CLED) MEDIA

PRODUCT:

Plate Media:

Cystine Lactose Electrolyte Deficient Agar	P1300
Cystine Lactose Electrolyte Deficient Agar With Andrades Indicator	P1306, P1307

PURPOSE:

CLED media are noninhibitory differential media used for the cultivation and presumptive identification of urinary tract pathogens.

PRINCIPLE:

CLED Agar was developed in 1960 by Sandys[5] and is deficient in electrolytes specifically to inhibit the swarming of *Proteus*. In 1965 the medium was modified by MacKey and Sandys[3] for use in urine culture by substituting lactose and sucrose for the mannitol and increasing the concentration of the agar and of the bromthymol blue indicator. In 1966 MacKey and Sandys[4] further modified the medium by the deletion of sucrose and the incorporation of cystine. The bromthymol blue indicator will change to yellow in the presence of lactose-fermenting bacteria. The cystine enhances the growth of cystine-dependant “dwarf colony” coliforms.

The formulation, modified by Bevis[1], replaced the bromthymol blue indicator with Andrades indicator (acid fuchsin) to achieve better differentiation between lactose fermenters and nonfermenters.

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) CLED Agar:

Beef Extract	3.0 g
Pancreatic Digest of Gelatin	4.0
Pancreatic Digest of Casein	4.0
Lactose	20.0
Agar	15.0
L-Cystine	128.0 mg
Bromthymol Blue	20.0

Final pH 7.3 ± 0.2 at 25°C

(2) CLED Agar With Andrades Indicator:

Same as (1) with 58.0 ml of Andrades Indicator[a].

Final pH 7.5 ± 0.2 at 25°C

[a]**Andrades Indicator:**

Acid Fuchsin	0.5 g
Sodium Hydroxide	16.0 ml

PRECAUTIONS:*

For in vitro diagnostic use only. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: *Shigella* species are inhibited due to the absence of electrolytes.

CLED With Andrades Indicator should not be incubated longer than 24 hours. Lactose fermenters, if present, may turn the entire plate pink after this time, masking the presence of nonfermenters.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and transported to the laboratory without delay.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Using a calibrated loop, inoculate the urine specimen onto the surface using standard microbiological procedures to obtain isolated colonies. Incubate aerobically at 35°C for 18-24 hours.

Interpretation:

COLONIAL MORPHOLOGY

Organism	CLED Agar	CLED Agar With Andrades Indicator
Escherichia coli	Yellow, clear, translucent colonies with granular surface.	Bright pink, clear colony with pink halo in surrounding media.
Proteus species	Blue-green, small, translucent colonies.	Blue, translucent.
Klebsiella/Enterobacter	Yellow to white, translucent, mucoid colonies.	Grey-green, mucoid.
Pseudomonas species	Green to blue-green colonies with matted surface and rough periphery.	Blue-green.
Staphylococcus aureus	Yellow, opaque colonies.	White to pale pink, opaque.
Staphylococcus epidermidis	White, opaque colonies.	White to pale pink, opaque.
Enterococcus faecalis and other enterococci	White, opaque raised colonies.	Deep yellow-orange.

Definitive identification can be made only after additional biochemical testing is performed.

Materials Required but Not Provided: Standard microbiological supplies and equipment are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC#):	Expected Results:
Staphylococcus aureus (25923)	Growth
Escherichia coli (25922)	Growth
Proteus vulgaris (8427)	Growth, no swarming
	Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Bevis, T. D., *J. Med. Technol.*, 25:38, 1968.
2. MacFaddin, J. F., *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, vol. 1, Williams and Wilkins, Baltimore, 1985.
3. MacKey, J. P., and G. H. Sandys, *Br. Med. J.*, 2:1286, 1965.
4. MacKey, J. P., and G. H. Sandys, *Br. Med. J.*, 1:1173, 1966.
5. Sandys, G. H., *J. Med. Lab. Technol.*, 17:224, 1960.

*For more detailed information, consult appropriate references.

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