Instructions For Use

ENGLISH

CLED Agar

Differential medium for detection of urinary pathogens.

INTENDED PURPOSE

Differential medium for the isolation, enumeration and differentiation of pathogenic bacteria in urine specimens. This medium is intended as an aid in the diagnosis, requiring further tests to complete the diagnostic results.

DESCRIPTION

CLED (Cysteine-, Lactose-, Electrolyte-Deficient) Agar is a medium for urine culture supporting the growth of urinary microorganisms and providing good colony differentiation while the swarming of *Proteus* is inhibited.

TYPICAL FORMULA*	(g/litre)
Enzymatic Digest of Gelatin	4.0
Enzymatic Digest of Casein	4.0
Beef Extract	3.0
Lactose	10.0
L-Cystine	0.128
Bromothymol Blue	0.02
Agar	15.0
Final pH 7.3 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Enzymatic digest of gelatin, enzymatic digest of casein and beef extract provide amino acids, nitrogen, carbon, vitamins and minerals required for organisms growth. Lactose is the fermentable carbohydrate. L-cystine is a growth supplement for cystine-dependent organisms. Bromothymol blue is the pH indicator changing color from green to yellow when lactose fermentation lowers the pH. Agar is the solidifying agent. Lack of electrolytes suppresses the swarming of *Proteus* and *Shigella* species.

PREPARATION

Dehydrated medium

Suspend 36.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Medium in bottles

Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, water bath, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Urine specimens should be sampled before antimicrobial therapy (where possible) and examined as soon as possible after collection. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Refer to specific guidelines for more information about specimen collection and preparation.

TEST PROCEDURE

Urine must be directly streaked over the agar surface no later than 2 h after collection or must be kept at 2-8°C (not longer than 24 h) to avoid microbial overgrowth.

Use calibrated loops (1 µl, 2µl or 10 µl) to inoculate the medium with the undiluted, well-mixed urine sample.

Incubate aerobically at 35 ± 2°C for 18-48 h.

For more details, consult appropriate guidance.

INTERPRETING RESULTS

After incubation, count the number of colonies on the plate.

If a 1µl loop is used, one colony equals 1000 CFU/ml (i.e., 1 x 10^6 CFU/litre). Using a 10 µl loop each colony corresponds to 100 CFU/ml of urine.

Observe the color and the morphology of the colonies for presumptive identification according to the following table.

Microorganism	**Typical Colony**
Escherichia coli	Yellow, opaque
Klebsiella, Enterobacter spp.	Yellow to whitish-blue, mucoid
Proteus spp.	Translucent blue
Salmonella spp.	Flat blue
Pseudomonas aeruginosa	Green, matt surface and rough periphery
Enterococcus spp.	Yellow, about 0.5 mm in diameter
Staphylococcus aureus	Deep yellow
Coagulase negative *staphylococci*	Pale yellow

Note: Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous**,** pale green or straw.
Appearance of Prepared Medium: Slightly opalescent, green to blue-green.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Criteria	Specification
Enterococcus faecalis	ATCC® 29212	50-100 CFU	18-24 h / 35 ± 2°C	Good growth ($P_R \geq 0.7$)	Yellow colonies
Escherichia coli	ATCC® 25922				Yellow colonies
Proteus mirabilis	ATCC® 12453				Blue colonies, no swarming
Proteus mirabilis	ATCC® 25933				Blue colonies, no swarming
Staphylococcus aureus	ATCC® 25923				Yellow colonies

A productivity ratio (P_R) of 0.7 is equivalent to a recovery rate of 70%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of CLED Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Due to nutritional variation, some strains may result in poor growth or fail to grow on this medium.

Observe aseptic techniques for urine specimen collection. Urine must be streaked directly onto the ground no later than 2 hours after harvesting or must be stored in the refrigerator (no longer than 24 hours) to avoid excessive growth of infectious agents or any contamination before inoculation of the medium.

CLED Agar is intended as an aid in the diagnosis of infectious diseases, requiring further tests to complete the diagnostic results. All identification tests should ideally be performed from non-selective agar.

WARNING AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD).**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
5) Do not use if the product or packaging appears to be damaged.
6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
9) Do not mix reagents of different batches. Please use the product within the validity period.
10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled
11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
CLED Agar	Plate 90 mm	20 plates	10026
		100 plates	10026*
	Bottle	6 x 100 ml	402180
		6 x 200 ml	412180
		6 x 500 ml	470110
	Dehydrated media	100 g	620012
		500 g	610012
		5 kg	6100125

Revision History

Revision	Release Date	Change Summary
1	2023-11-08	Updated layout and content in compliance with IVDR 2017/746

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds