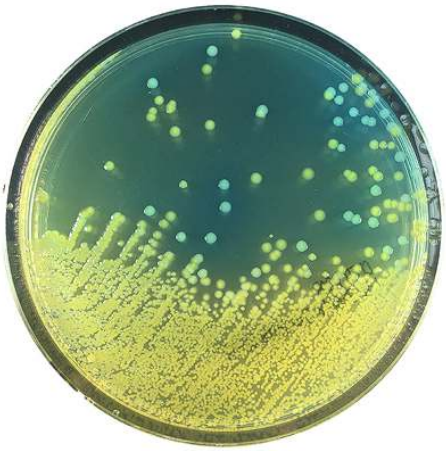
INSTRUCTIONS FOR USE

.

CLED MEDIUM

Dehydrated culture medium



CLED Medium:
Lactose fermenting *E.coli* (yellow colonies) and lactose non-fermenting *Salmonella* (blue colonies)

1 - INTENDED USE

In vitro diagnostic. Differential culture medium for isolation, enumeration and presumptive identification of microorganisms from urine.

2- COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	4.000 g
Pancreatic digest of gelatin	4.000 g
Peptone	3.000 g
Lactose	10.000 g
L-cystine	0.128 g
Bromothymol blue	0.020 g
Agar	15.000 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

CLED Medium, is based on the "electrolyte deficient medium" first described by Sandys[1] for urinary bacteriology to prevent the swarming of *Proteus* spp and later modified by Mackey and Sandys[2] by replacing the mannitol with 1% lactose and 0.2% sucrose and increasing the pH indicator and the agar concentrations. Mackey and Sandys[3] further modified the medium by the incorporation of L-cystine in order to enhance the growth of cystine-dependent "dwarf colony" coliforms and by deletion of sucrose. This final Cystine - Lactose - Electrolyte – Deficient (C.L.E.D.) medium is found to be ideal for the dip-slide inoculation and for urinary bacteriology in general, good colonial differentiation and easy recognition being particular features.[3] It supports the growth of all urinary potential pathogens. It also supports the growth of a number of essential contaminants such as diphtheroids, lactobacilli, and micrococci, which gives an indication of the extent of the contamination, and whilst being non-inhibitory it prevents the swarming of *Proteus* sp.[3]
Animal peptones provide carbon, nitrogen, vitamins and trace elements for microbial growth; cystine enhance the growth of "dwarf colony" coliforms[3] ; lactose is present in the medium as a fermentable carbohydrate: lactose-fermenting bacteria acidify the medium with a colour change of bromothymol blue from blue-green to yellow.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 36.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green-grey, fine, homogeneous, free-flowing powder
Medium appearance of solution and plates	blue-green, limpid
Final pH at 20-25 °C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
CLED Medium	Dehydrated medium	40129012 40129014	500 g (13.8L) 5 kg (138L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

CLED Medium is intended for the microbiological processing of clinical specimens such as urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[4,5]

9- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Mix the urine gently to avoid foaming. Dip the end of a sterile calibrated loop (e.g. 1µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank. Use this to inoculate CLED Medium plate from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back-and-forth fashion. The inoculum of urine is spread over the entire agar surface to simplify counting of colonies after growth.
Incubate at 35-37°C in air for 24 to 48 hours.
Although most urinary tract pathogens grow readily, slowly growing pathogens and those inhibited by the presence of antimicrobials in the patient specimen may not appear after overnight incubation (16 h). Perform leukocyte esterase and nitrite tests to determine which

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milano, Italia
Tel. +39 02 25209.1
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

cultures get incubated for a full 48 hours. Urine cultures that are negative after overnight incubation but had one or both positive enzyme tests should be incubated for an additional day or re-inoculated on a blood agar plate.[4]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth, count the number of colonies (CFU) on the plate and record the specific morphological, chromatic, characteristics of the colonies.
If a 1µL loop is used, one colony equals 1000 CFU/mL, if a 10µL loop is used, one colony equals 100 CFU/mL.
Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of $\geq 10^5$ CFU/mL are indicative of an infection and counts below this usually indicate contamination.[5]
In specific patient groups, counts between 10^5 CFU/mL and 10^2 CFU/mL may be significant; a pure isolate with counts between 10^4 and 10^5 CFU/mL should be evaluated based on clinical information or confirmed by repeated culture.[5] For urine collected by suprapubic bladder puncture any CFU detected indicates an infection.[5]
Consult appropriate references for complete interpretation criteria of the microbial count.[4,5]

Typical colonial morphology on CLED Medium is as follows[6]:

Escherichia coli	Yellow, opaque colonies, centre slightly deeper yellow
Klebsiella/Enterobacter	Yellow to whitish-blue colonies, mucoid
Salmonella	Blue, flat colonies
Proteus	Blue, translucent colonies
Pseudomonas	Green colonies, with typical rough (matted) surface and periphery
Enterococci	Small yellow colonies
Staphylococcus aureus	Deep yellow colonies, uniform in colour
Staphylococcus epidermidis	Pale yellow colonies, more opaque than *S.aureus*

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.aureus	ATCC 25923	35-37°C / 18-24H / A	good growth, yellow colonies
E.coli	ATCC 25922	35-37°C / 18-24H / A	good growth, yellow colonies with yellow halo
P.vulgaris	ATCC 8427	35-37°C / 18-24H / A	good growth, bluish colonies not swarmed

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated CLED Medium is tested for productivity by comparing the results with a previously approved Reference Batch and with Tryptic Soy Agar.
Productivity is tested by a quantitative test with the target strain *E.faecalis* ATCC 19433; CLED Medium plates are inoculated with decimal dilutions in saline of a colonies suspension and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on CLED and TSA plates and the productivity ratio is calculated ($Pr = CFU_{CLED}/CFU_{TSA}$). If *Pr* is ≥ 0,5 and if the colonies morphology and colour are typical (yellow colonies) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains: *E.coli* ATCC 25922, *P.vulgaris* ATCC 9484, *P.mirabilis* ATCC 10005, *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736, S.*aureus* ATCC 25923, *C.albicans* ATCC 18804. After incubation, the colonies and medium colour and the amount of growth is evaluated and recorded. All strains show a good growth with typical colours.

13 - LIMITATIONS OF THE METHOD

- Medium is basically non-selective but, due to electrolyte exclusion, *Shigella* spp. usually do not grow on the medium.[6]
- If in the specimen the presence of genitourinary pathogens such as *Neisseria gonorrhoeae, Gardnerella vaginalis, Ureaplasma* is suspected, specific culture media must be inoculated.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.

- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

15 - REFERENCES

1. Sandys GH. A new method of preventing swarming of *Proteus* sp with a description of a new medium suitable for use in routine laboratory practice. J Med Lab Technol 1960;17:224-233
2. Mackey JP, Sandys GH. Laboratory diagnosis of infection of the urinary tract in general practice by means of a dip-inoculum transport medium Br Med J 1965; 2:1286-1288
3. Mackey JP, Sandys GH. Correspondence. Diagnosis of urinary infections. Br Med J 1966; 1:1173
4. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
5. Public Health England UK Standards for Microbiology Investigations. Investigation of urine. Bacteriology, B 41, 2019
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/05
Revision 3	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/02
Revision 4	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.