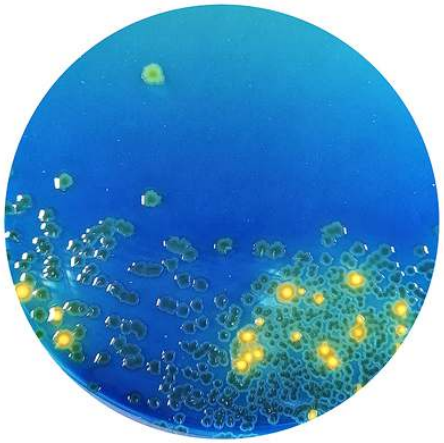
INSTRUCTIONS FOR USE

DRIGALSKI LACTOSE AGAR

Dehydrated culture medium



Drigalski Lactose Agar: *E.coli* (yellow colonies), S.Enteritidis (greenish colonies)

1 - INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation of *Enterobacteriaceae* and other Gram negative bacilli from clinical and non clinical specimens.

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

Peptone	15 g
Beef extract	3 g
Yeast extract	3 g
Sodium desoxycholate	1 g
Sodium thiosulphate	1 g
Lactose	15 g
Agar	13 g
Crystal violet	5 mg
Bromothymol blue	80 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Wilhelm von Drigalski and Heinrich Conradi in 1902 discovered the inhibitory activity of the crystal violet on the growth of Gram positive bacteria and developed a selective and differential medium for the isolation of B.typhi. Drigalski-Conradi medium, modified several times over the years, is the formulation on which the current Drigalski Lactose Agar is based, still widely used as a selective and differential medium for the isolation of *Enterobacteriaceae* and other non-fermenting Gram negative bacteria from clinical specimens such as urine, faeces, and other biological materials.[1]

The use of Drigalski Lactose Agar, with the addition of cephalosporins, has been proposed for the isolation of extended spectrum beta-lactamase-producing enterobacteria,[3] and, supplemented with carbapenems, for the isolation of carbapenemase-producing enterobacteria[4].

Peptone, meat extract and yeast extract provide nitrogen, carbon, minerals and vitamins for the microbial growth The selective compounds of the medium are sodium desoxycholate, crystal violet and sodium thiosulphate which have an inhibitory activity against Gram positive bacteria; 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 51 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 115°C for 15 minutes. Cool to 47-50 °C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, limpid
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Drigalski Lactose Agar CND: W0104010101; EDMA:14.01.01.01; RDM:1867770/R	Dehydrated medium	4013302	500 g (9,8L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and 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

Drigalski Lactose Agar is intended for the bacteriological processing of clinical specimens such as urine, faeces, and other biological materials. Good laboratory practices for collection, transport and storage of the specimens should be applied.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic conditions at 35-37°C for 18-24 hours.

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10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Gram negative bacteria grow with different characteristics depending on their ability to ferment lactose and to induce the pH indicator changes.
E. coli, Klebsiella, Citrobacter, Enterobacter ferment lactose with acid production and grow with yellow or yellow-green colonies with, often, an opaque yellow halo.
Salmonella, Shigella, Proteus, Alkaligenes, Pseudomonas do not ferment lactose and grow with gray to green-blue colonies.

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, it is responsibility of the end-user to perform Quality Control testing 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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Escherichia coli ATCC 25922	35-37°C / 18-24 H / A	growth, yellow colonies
*S.*Enteritidis NCTC 5188	35-37°C / 18-24 H / A	growth, blue-green colonies
S.aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection, NCTC: National Type Culture Collection of the UK Health Protection Agency

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Drigalski Lactose Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with 7 Gram negative target strains: *E.coli* ATCC 25922, *E.aerogenes* ATCC 13048, *C.freundii* ATCC 8090, *A.calcoaceticus* ATCC 19606, *S.*Enteritidis NCTC 5188, *P.mirabilis* ATCC 10005. After incubation the colonies' colours and characteristics and the amount of growth is evaluated and recorded. All strains grow with typical colonies' colours and the amount of growth is comparable in both batches.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target Gram positive strains *E.faecalis* ATCC 19433 and *S.aureus* ATCC 25823. The growth of non-target strains is inhibited at the dilution 10^{-1}.

13 - LIMITATIONS OF THE METHOD

- On this medium, some *Proteus* strains may grow with swarming colonies.
- Incubations prolonged beyond 24 hours can induce a medium change to alkalinity of the lactose-positive strains.
- 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 relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; 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 Material Safety Data Sheet.
- 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 does not 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 S.r.l. 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 inoculated plates 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 are available on the website www.biolifeitaliana.it.
- 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. This also applies in relation to any third party rights. 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 +2°C /+8°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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - REFERENCES

1. Dupeyron CM, Guillerman GA, Leluan GJ. Rapid diagnosis of gram negative urinary infections: identification and antimicrobial susceptibility testing in 24 hours. J Clin Pathol 198; 39:208-211.
2. Grohs P, Tillecovidin B, Caumont-Prim A, et al. Comparison of Five Media for Detection of Extended-Spectrum Beta-Lactamase by Use of the Wasp Instrument for Automated Specimen Processing. J Clin Microbiol 2013; 51: 2713–2716.
3. Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP. Faecal Carriage of Multidrug-Resistant Gram-negative Bacilli During a Non-Outbreak Situation in a French University Hospital. J Antimicrob Chemother 2010; 65: 2455-8.
4. Nordmann P, Girlich D, Poirel L. Detection of Carbapenemase Producers in Enterobacteriaceae by Use of a Novel Screening Medium. J Clin Microbiol 2012; 50: 2761–2766.

TABLE OF APPLICABLE SYMBOLS

REF o 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 1	Updated layout and content	2020/05

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

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