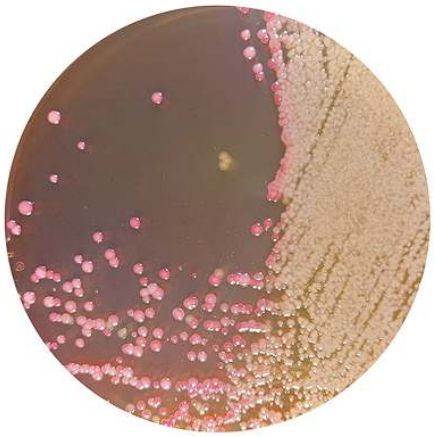
INSTRUCTIONS FOR USE

DESOXYCHOLATE AGAR

Dehydrated culture medium



Desoxycholate Agar: *K.pneumoniae* (pink-red colonies), *S.*Enteritidis (cream colonies)

1 - INTENDED USE

In vitro diagnostic. Moderately selective medium for the isolation and differentiation of Gram-negative enteric bacteria from clinical specimens and for the enumeration of coliforms in dairy products.

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

Peptocomplex	10.000 g
Lactose	10.000 g
Sodium chloride	5.000 g
Potassium phosphate bibasic	2.000 g
Ferric citrate	1.000 g
Sodium citrate	1.000 g
Sodium desoxycholate	1.000 g
Neutral red	0.033 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

Desoxycholate Agar, prepared on the basis of the formulation described by Leifson[1] in 1935, is intended for the isolation and differentiation of Gram negative enteric bacteria from a variety of clinical samples[2] and for the enumeration of coliforms in dairy products[3].

Peptocomplex provides nitrogen, carbon and trace elements for microbial growth; potassium phosphate acts as a buffer system, sodium chloride maintains the osmotic balance; the moderate selectivity of the medium is due to the presence of sodium desoxycholate, sodium citrate and iron citrate which allow a good growth of Gram negative bacteria and a partial to total inhibition of Gram positive bacteria. Lactose is included into the medium as a fermentable carbohydrate, neutral red as a pH indicator: coliforms ferment lactose producing an acidification of the medium around the colony highlighted by the precipitation of sodium desoxycholate and the development of a red colour; bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 45 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, cool to 47-50 °C, mix well and pour into sterile Petri dishes. Do not sterilize in autoclave and do not overheat.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige to pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Desoxycholate Agar ;	Dehydrated medium	4013702	500 g (11.1L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

Desoxycholate Agar is intended for the bacteriological processing of clinical specimens such as faeces in which detect Gram negative enteric bacteria. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food samples, refer to the applicable international standards.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Clinical specimens[4]

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.

Enumeration of coliform bacteria in dairy products[3] :

- Introduce 1-4 ml of sample (or decimal dilutions of the sample) into sterile Petri dishes.
- Add 10-20 ml of medium cooled to 47-50°C and mix well the medium with the inoculum.
- Allow the medium to solidify and pour a surface covering layer of 3-4 ml of uninoculated medium.

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- Incubate aerobically at 35-37°C for 18-24 hours.

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.
Lactose non fermenting Gram-negative enteric bacteria: colourless colonies
Lactose fermenting Gram negative enteric bacteria (coliforms): red colonies sometimes surrounded by a red-pink opaque zone (precipitation of sodium desoxycholate).

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, red-violet colonies
*S.*Enteritidis ATCC 13076	35-37°C / 18-24 H / A	growth, colourless colonies
E.faecalis ATCC 29212	35-37°C / 18-24 H / A	inhibited

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 Desoxycholate Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative eometric technique, by incubating at 35-37°C for 18-24 hours, with 7 Gram negative strains: *E.coli* ATCC 25922, *E.coli* ATCC 8739, *E.aerogenes* ATCC 13048, *S.*Enteritidis ATCC 13076, *P.vulgaris* ATCC 8427, *P.aeruginosa* ATCC 14207. After incubation the colonies' colours and characteristics and the amount of growth is evaluated and recorded. All strains grow with typical colonies 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 29212 and *S.aureus* ATCC 25923. The growth of non-target strains is inhibited at the dilution 10^{-1} .

13 - LIMITATIONS OF THE METHOD

- Aerobic or facultative anaerobic Gram-negative bacteria other than *Enterobacteriaceae* (e.g. *Pseudomoas*, *Aeromonas*) may grow on the medium with colourless colonies.
- The medium does not differentiate non-fermenting lactose bacteria such as *Proteus* spp. from *Salmonella* and *Shigella*. It is advised to screen the colonies by flooding the plate with one drop of MUCAP reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[10]
- Desoxycholate Agar does not contain an indicator system for the production of hydrogen sulphide therefore *Salmonella* colonies do not have a black centre.
- At pH above 7.5 the medium loses some of its inhibitory properties towards Gram positive bacteria.[1,4]
- With overheating there may be a decrease in the gelling power of the agar due to its hydrolysis by iron citrate and sodium citrate.[1,4]
- It is advisable to use a freshly prepared medium.[4]
- 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 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 media 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/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Leifson E. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J Pathol Bacteriol 1935; 40: 581-599.
2. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.335.
3. American Public Health Association (1978) `Standard Methods for the Examination of Dairy Products. 14th ed. 1978; New York: APHA Inc, pp. 58-59.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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
Revision 2	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/03
Revision 3	Removal of obsolete classification	2023/04

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