INSTRUCTIONS FOR USE

DESOXYCHOLATE CITRATE AGAR

Dehydrated culture medium

Salmonella Typhimurium
on Desoxycholate Citrate Agar

1 - INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella,* from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptone	5.00 g
Beef extract	5.00 g
Lactose	10.00 g
Sodium citrate	5.00 g
Ferric ammonium citrate	1.00 g
Sodium deoxycholate	2.50 g
Sodium thiosulphate	5.00 g
Neutral red	0.03 g
Agar	15.00 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 Citrate Agar, prepared on the basis of a modification of the formula described by Leifson[1] in 1935, is intended for the isolation and differentiation of Gram-negative enteric pathogens (*Salmonella* and *Shigella*) from faecal specimens.
Several formulas have been proposed, based on the original media of Leifson[1], Haynes[2], Hajna and Damon[3] with different concentrations of sodium citrate, ferric citrate or ferric ammonium citrate and sodium desoxycholate, presence or absence of sucrose and sodium thiosulfate, slightly different concentrations of neutral red. Desoxycholate Citrate Agar (Leifson) has more selectivity characteristics than Desoxycholate Agar (REF 401370) and is less selective than Haynes' medium.[4]
Peptone and beef extract provide carbon, nitrogen and trace elements for bacterial growth; the selectivity of the medium is due to the presence of sodium desoxycholate, sodium citrate and ferric citrate, which allow a good growth of Gram-negative bacteria, a partial inhibition of coliforms and a total inhibition of Gram-positive bacteria. Lactose is included as a fermentable carbohydrate, neutral red as a pH indicator; bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies. The coliform strains, that are able to grow in the presence of selective compounds, ferment lactose producing an acidification of the medium around the colonies, the precipitation of sodium desoxycholate and the development of a red colour. *Salmonella* spp. produce thiosulphate reductase that causes the release of a sulphide molecule from the sodium thiosulfate present in the medium; this sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 48.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. 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	pink, fine, homogeneous, free-flowing powder
Medium appearance	red, limpid
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Desoxycholate Citrate Agar	Dehydrated medium	4013752	500 g (10.3L)

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 Citrate Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab. Good laboratory practices for collection, transport and storage of clinical 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.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using the enrichment step in Selenite Broth followed by subculture on Desoxycholate Citrate Agar and on a second plating medium.[5]
For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture in two different selective media: Desoxycholate Citrate Agar and a second less selective medium (e.g., Mac Conkey Agar).[5]

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

Incubate inoculated Desoxycholate Citrate Agar plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours. If there is no microbial growth or doubts in reading the black colony centre, incubate for an additional 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Lactose non-fermenting Gram negative enteric bacteria: colourless colonies with or without black centre. *Salmonella* colonies generally have a light black centre, *Shigella* colonies do not have a black centre.
Lactose fermenting Gram negative enteric bacteria (coliforms): red colonies sometimes surrounded by a red-pink opaque zone.

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
S.Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, red colonies with light black centre
S.flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
E.faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E.coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, red colonies

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 Citrate 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 4 target strains: *S*.Enteritidis NCTC 5188, *S*.Typhimurum ATCC 14028, *S*.Gallinarum clinical isolate, *S.flexneri* ATCC 12022, *S.sonnei* ATCC 9290, *S.boydii* ATCC 9207. *Salmonella* colonies are colourless with light black centre, *Shigella* colonies are colourless; the amount of growth on the plates is evaluated and shall be comparable in both batches.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target Gram-positive strains *E.faecalis* ATCC 19433 and *S.aureus* ATCC 25923 and non-target Gram-negative *P.mirabilis* ATCC 10005, and *E.coli* ATCC 25922. The growth of Gram positive strains is totally inhibited, the growth of non target Gram-negative strains is partially inhibited and the colonies show typical chromatic characteristics, according to the specifications.

13 - LIMITATIONS OF THE METHOD

- If pH is raised above 7.5 many Gram-positive bacteria may exhibit growth.[4]
- With overheating there may be an increased degree of inhibition and a decrease in the gelling power of the agar due to its hydrolysis by ferric citrate and sodium citrate.[1,4]
- It is recommended to use freshly prepared medium.[4]
- Do not remelt the medium once it has been prepared as this can reduce its productivity characteristics towards target strains.[6]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella*, with lower selectivity such as Mac Conkey Agar or Levine EMB Blue Agar and with higher selectivity such as SS Agar or, if S.Typhi is suspected, Bismurh Sulphite Agar, should be used; other media for the isolation of other enteric pathogens should be inoculated with the specimen.[5]
- Non-enteric, lactose non-fermenting organisms such as *Pseudomonas* and *Aeromonas* may grow.
- Surface colonies of Lactose non-fermenting bacteria often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms.[4]
- Some strains of *Proteus* spp. may not be completely inhibited and colonies may resemble *Salmonella*. It is advised to screen the colonies by adding 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.[7]
- 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 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.
- 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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).

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. Hynes M. The isolation of intestinal pathogens. J Pathol Bacteriol 1942; 54:193-207.
3. Hajna AA, Damon SR, New enrichment and plating medium for the isolation of Salmonella and Shigella organisms. App Microbiol. 1956; 4:341.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.685.
6. Liang C, Fung DYC. Performance of Some Heat-Sensitive Differential Agars Prepared and Melted by Microwave Energy. J Food Prot. 1988 Jul; 51(7):577-578.
7. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.

401375 DESOXYCHOLATE CITRATE AGAR

SDS
Regulation (EU) 2020/878

Contains: SODIUM DEOXYCHOLATE
IRON III AMMONIUM RED CITRATE
Classification: none
Labelling none
Hazard statements: **EUH210** Safety data sheet available on request.

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 “performances characteristics”, “limitation of the method”, “precautions and warnings” and “storage conditions and shelf life”	2022/03
Revision 3	Removal of obsolete classification	2023/04
Revision 4	Insert SDS’s section	2025/05

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