INSTRUCTIONS FOR USE

SERUM TELLURITE AGAR

Ready-to-use plates

C.diphtheriae on Serum Tellurite Agar

1 - INTENDED USE

In vitro diagnostic device. Selective and differential medium for the isolation and detection of *Corynebacterium diphtheriae* from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	15 g
Papaic digest of soy bean meal	5 g
Sodium chloride	5 g
Agar	15 g
Horse serum	50 mL
Potassium tellurite, 1% solution	10 mL
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Serum Tellurite Agar is a modification of Hoyle Medium[1], described in the Manual of Bacteriological Techniques of the Regional Committee for the Organization of Pathology Services[2]. Serum Tellurite Agar is a selective and differential medium for the isolation and detection of *Corynebacterium diphtheriae*, in clinical specimens.[2] The medium contains two peptones as sources of nitrogen and carbon, necessary for microbial growth. Sodium chloride provides essential electrolytes and contributes to the osmotic balance. Horse serum stimulates the growth of corynebacteria and potassium tellurite inhibits the growth of most normal Gram-negative bacteria of the upper respiratory tract[3]; it is reduced by corynebacteria and other microorganisms with the formation of grey or black colonies.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.1

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Serum Tellurite Agar	Ready-to-use plates	549998	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Serum Tellurite Agar plates can be directly inoculated with clinical specimens or with culture obtained on Loeffler's medium. In case of respiratory diphtheria, material for culture should be obtained on a swab (either cotton or polyester tipped swab) from the inflamed area of nasopharynx; if membranes are present and can be removed, they should also be sent to the laboratory.[4] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information. Collect specimens before antimicrobial therapy where possible.

8- TEST PROCEDURE

Allow plates to come to room temperature. Roll the swab with the specimen or with the sub-culture from Loeffler medium on a restricted area of the Serum Tellurite Agar plate, then streak with a loop on four quadrants, to disperse the inoculum and obtain isolated colonies. Incubate aerobically at 37°C for 24-48 hours, but discarding the plates as negative after 4 days of incubation. Observe daily for the development of typical colonies.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. After 24-48 hours of incubation *C.diphtheriae* cultivates with grey-black colonies with light halo and jagged edges, 1 to 5 mm in diameter, with often rough and sometimes smooth surfaces (forms RS). The greyish colour of the colonies intensifies with the prolongation of the incubation until it reaches grey-black in the 4th day.

10 - 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]

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CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C.diphtheriae ATCC 11913	35-37°C / 24-48 H / A	growth, grey colonies
E.coli ATCC 25922	35-37°C / 24-48 H / A	inhibited

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

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to use plates of Serum Tellurite Agar is tested for productivity and selectivity.
Productivity is tested by semi-quantitative ecometric technique with the target strains *C.diphtheriae* ATCC 11913 and *C.diphtheriae* ATCC 13812. After incubation at 35-37°C for 24-48 hours in aerobic atmosphere, *C.diphtheriae* shows a good growth with typical grey colonies.
Selectivity is evaluated by semi-quantitative ecometric technique and 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 organisms *E.coli* ATCC 25922 and *S.aureus* ATCC 25923. After incubation at 35-37°C for 44-48 hours in aerobic atmosphere, the growth of *E.coli* is inhibited while *S.aureus* grows with black colonies.

12 - LIMITATIONS OF THE METHOD

- Tellurite inhibits the growth of many non-coryneform bacteria but even a few *C.diphtheriae* strains are sensitive to potassium tellurite and therefore do not grow on Serum Tellurite Agar.[4]
- The growth on Serum Tellurite Agar and the reduction of tellurite are not specific for *C.diphtheriae* since many other coryneforms and other Gram-positive bacteria may also produce black colonies.[4]
- The medium is not inhibitory to Gram-positive bacteria: pseudodiphtheria, staphylococci, streptococci, micrococci, listeriae can grow with white-grey-black colonies. *Candida* grows with small greyish-white colonies.[2]
- It is advisable to inoculate, together with Serum Tellurite Agar, other plated or tubed media such as Blood Agar and Loeffler's Medium.[2,3,4]
- 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 required and 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 the microscopic and/or other diagnostic tests.

13 - 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.
- This product is not classified as dangerous according to current European legislation.
- 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 the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- 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.
- The Certificates of Analysis and the Safety Data Sheet of the product 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. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Hoyle L. A tellurite blood-agar medium for the rapid diagnosis of diphtheria. Lancet, 1941;1:175
2. Comitato Regionale per l'Ordinamento dei Servizi di Patologia (1977) Manuale di Tecniche Batteriologiche. Giunta Regionale della Lombardia Ass. Sanità.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Bernard KA. Coryneform Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

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	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/12
Instructions for Use (IFU) - Revision 2	Removal of the obsolete classification	2023/03

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

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