INSTRUCTIONS FOR USE

TRYPTIC SOY AGAR

Dehydrated culture medium

Bacillus cereus on Tryptic Soy Agar

1 - INTENDED USE

In vitro diagnostic. General purpose medium for cultivation and maintenance of non-fastidious and moderately fastidious microorganisms. For microbial enumeration of non-sterile pharmaceutical products and cosmetics. Supplemented with defibrinated animal blood, Tryptic Soy Agar is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of haemolytic properties.

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

Pancreatic digest of casein	15 g
Soy peptone	5 g
Sodium chloride	5 g
Agar	15 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

Tryptic Soy Agar (TSA) is one of the most widely used culture media in clinical and industrial microbiology. TSA has a multitude of uses in clinical and non-clinical laboratories including isolation, cultivation and purification of colonies of non-fastidious and moderately fastidious microorganisms and maintenance of stock cultures.[1] As it doesn't contain the X and V factors, it is suitable for identification of *Haemophilus* sp. by adding on the agar surface discs or strips impregnated with X (Hemin) and V (NAD) factors.[2] It is recommended as a reference medium, when testing selective media, to measure the degree of inhibition.[3] TSA is the medium specified as "casein soya bean digest agar" in the harmonised EP, USP JP method[3] for microbial enumeration of non-sterile pharmaceutical products. It is recommended by ISO Standard 21149 for the enumeration and detection of aerobic mesophilic bacteria in cosmetics[5].

Tryptic Soy Agar may be supplemented with defibrinated animal blood (at concentrations between 5% and 7%) to provide a more nutritious medium for the growth of fastidious organisms; the addition of animal blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.

TSA may be supplemented with 0.7g/L lecithin and 5g/L Polysorbate 80, which neutralise the activity of quaternary ammonium compounds and other disinfectants, for determining the efficacy of sanitization of products, sanitary areas, containers.[6]

Tryptic Soy Agar with the addition of salt can be helpful in determining the halotolerance level of microorganisms.[6]

Tryptic Soy Agar is prepared with selected casein and soy peptones: the combination of casein and soy peptones renders the medium nutritious by supplying organic nitrogen in the form of amino acids and polypeptides. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 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.

Notes. The medium may be also dispensed in tubes before sterilisation and cooled in slanted position. For the preparation of blood plates, to the medium autoclaved and cooled to 47-50°C, add 5-7% defibrinated animal blood, mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Soy Agar	Dehydrated medium	4021502	500 g (12,5 L) CND: W0104010101; EDMA:14.01.01.01; RDM: 1875795/R
		4021504	5 kg (125 L) CND: W0104010101; EDMA:14.01.01.01 RDM: 1875803/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Un-supplemented Tryptic Soy Agar should not be used for the direct inoculation of clinical specimens. Generally, TSA is used for the sub-culture of microorganisms grown on other culture media. Non-clinical samples analyzed with Tryptic Soy Agar include non-sterile pharmaceutical products and cosmetics. Refer to the quoted literature for sample collection and preparation.[4,5]

If supplemented with animal blood, the poured plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens, related to specific infections.[7-9] Collect

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

specimens before antimicrobial therapy where possible and apply good laboratory practices for collection, transport and storage of the clinical specimens; consult appropriate references for further information.[7]

9 - TEST PROCEDURE

Allow plates or tubes to come to room temperature and to dry the surface of the medium.
For the subculture of colonies, by means of a sterile needle or loop, inoculate an un-supplemented TSA plate with a colony cultivated on another isolation medium. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the inoculated organism and the local applicable protocols.
When using TSA supplemented with defibrinated animal blood, streak the clinical 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 at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 18-24, 48 and if necessary, 72 hours.
For the microbial enumeration in non-sterile pharmaceutical products and cosmetics consult the references.[4,5]

10 - READING AND INTERPRETATION

After incubation, the presence of microorganisms is indicated by the appearance of colonies of various morphology and size on the un-supplemented medium surface. The characteristics of the growth are closely related to the type or types of cultivated microorganisms.
By cultivation on sheep blood agar plates prepared with Tryptic Soy Agar, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

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 of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ t / ATM	EXPECTED RESULTS
S.aureus	ATCC 25923	35-37°C / 18-24H / A	good growth
E.coli	ATCC 25922	35-37°C / 18-24H / A	good growth

User quality control of TSA used for microbial enumeration in non-sterile pharmaceutical products and cosmetics should meet the requirements of EP[4] and ISO Standard[5]
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 Tryptic Soy Agar (Test Batch: TB), un-supplemented and supplemented with defibrinated sheep blood, is tested for productivity and haemolytic pattern by comparing the results with a previously approved Reference Batch (RB).
Productivity of un-supplemented TSA is tested by a quantitative test with the following strains: *P.aeruginosa* ATCC 9027, *E.coli* ATCC 25922, *B.cereus* ATCC 11778, *B.subtilis* ATCC 6633, *S.aureus* ATCC 6538, *S.aureus* ATCC 25923, *L.monocytogenes* ATCC 13932, *C.albicans* ATCC 10231, *A.brasiliensis* ATCC 16404. Tryptic Soy Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 30-35°C for 24-72 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr = CFU_{TB}/CFU_{RB}$) is calculated. If *Pr* is ≥ 0,7 and if the colonies morphology is typical, the results are considered acceptable and conform to the specifications.
Productivity of TSA supplemented with 5% defibrinated sheep blood is tested by semi-quantitative ecometric technique with the following strains: *S.pyogenes* ATCC 19615, *S.agalactiae* ATCC 12386, *S.aureus* ATCC 25923. After incubation at 35-37°C for 18-24 hours the types of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth with typical haemolytic pattern.

13 - LIMITATIONS OF THE METHOD

- When using blood supplemented TSA, depending on the specimens analyzed and the microorganisms being tested for, for the examination of clinical specimens, it is recommended to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[10]; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic 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.
- 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 preparation process of plated or tubed or bottled 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.
- 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 period of validity of the finished products, according to the type (plates/tubes/bottles), the added supplements and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
2. Ledeboer NA, Doern GV. Haemophilus. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.667.
3. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media
4. European Pharmacopoeia, current edition
5. ISO 21149:2017. Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. 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.
8. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
9. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
10. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

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 1	Updated layout and content	2020/06
Revision 2	Modification of “intended use”, “precautions and warnings” and “storage conditions and shelf life”.	2021/11

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it