Trypticasein Soy Agar (TSA) EP/USP/ISO

Cat. 1068

For general bacteriology methods and the determination of hemolytic reactions

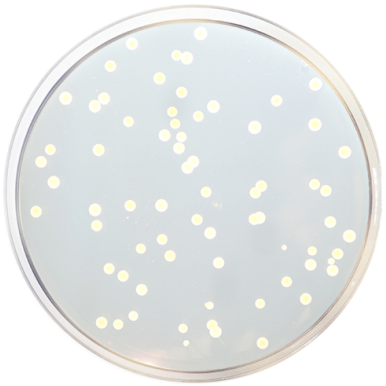
Practical information

Aplications	Categories
Non selective enumeration	General use

Industry: Water / Clinical / Food / Cosmética / Final product Quality Control

Regulations: USP / ISO 11133 / ISO 11930 / ISO 18415 / ISO 18416 / European Pharmacopoeia / ISO 21149 / ISO 21150 / ISO 22717 / ISO 22718 / ISO 9308

CE IVD



Principles and uses

Trypticasein Soy Agar (TSA) is a general purpose medium very rich in nutrients for general use in microbiological laboratories and for the cultivation and isolation of fastidious or non fastidious microorganisms, or for the maintenance of stock culture. It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, Neisseria, etc. from clinical samples.

Containing two peptones as rich nitrogen sources, obtained by the enzymatic hydrolysis of casein and soy proteins, this medium supports the growth of a great variety of microorganisms, including fastidious aerobes and anaerobes. Soy peptone also contains natural sugars which promote bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

Since it lacks carbohydrates it is very useful in the study of hemolytic reactions and also in the preparation of chocolate agar.

If desired, antibiotics can easily be incorporated as well as other supplements or inhibitory agents.

Some of the microorganisms that grow on this medium are the following: Streptococcus, Neisseria, Brucella, Corynebacteria, Listeria, Pasteurella, Vibrio, Haemophilus vaginalis, Candida, etc.

The European Pharmacopoeia, USP recommends this medium in the paragraph 2.6.12: “Microbiological examination of non – sterile products: Microbial enumeration test” for the examination of TAMC and TYMC in products.

The ISO 11133 Norm recommends the Trypticasein Soy Agar (TSA) as a reference medium.

Formula in g/L

Bacteriological agar	15	Pancreatic digest of casein	15
Sodium chloride	5	Papaic digest of soyabean meal	5

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 40 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 °C for 15 minutes. Large quantities may require a longer sterilization time, but the temperature should not be increased.

To prepare blood plates for hemolysis studies, add 5 - 10% of defibrinated sterile blood to the sterile medium, previously cooled to 45°C. Be careful to avoid bubble formation when adding the blood to the cooling medium and rotate the flask or bottle slowly to create a homogeneous solution.

Instructions for use

» For clinical diagnosis, the type of samples is blood.

- Streak in parallel the surface of the plate with a loop or swab.
- Incubate in aerobic conditions at 35±2 °C for 18-72 hours.
- Reading and interpretation of results.

» For other uses not covered by the CE marking:

Examination of TAMC and TYMC in products according to European Pharmacopoeia:

Membrane filtration:
- Prepare the product sample suspending, dissolving or diluting the product to be examined in the Trypticasein Soy Broth.
- Transfer the appropriate amount of the sample to a membrane filter.
- Place the membrane to the surface of Trypticasein Soy Agar (Cat. 1068) in case of TAMC or Sabouraud Dextrose Agar (Cat. 1024) in case of TYMC.
- Incubate the plate of Trypticasein Soy Agar (Cat. 1068) at 30-35 ºC for 3-5 days and the plate of Sabouraud Dextrose Agar (Cat. 1024) at 20-25 ºC for 5-7 days.

Plate-count methods:
- Prepare the product sample suspending, dissolving or diluting the product to be examined in the Trypticasein Soy Broth.
- Inoculate the plates of Trypticasein Soy Agar (Cat. 1068) in case of TAMC or Sabouraud Dextrose Agar (Cat. 1024) in case of TYMC, conforming to the pour-plate method or the surface-spread method.
- Incubate the plates of Trypticasein Soy Agar (Cat. 1068) at 30-35 ºC for 3-5 days and the plates of Sabouraud Dextrose Agar (Cat. 1024) at 20-25 ºC for 5-7 days.
- Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 (TAMC) or 50 (TYMC).

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber / Cherry-red with blood	7,3 ± 0,2

Microbiological test

According to European Pharmacopoeia. Total aerobic microbial count (TAMC); Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 and Aspergillus brasiliensis ATCC 16404:
Incubation conditions: (30-35 ºC / <=3 days: bacteria /<=5 days: fungi).
Inoculation conditions: (<=100 CFU).

According to ISO 11133 Food Microbiology:
Incubation conditions:
Bacillus cereus ATCC 11778 according to ISO 7932 (30±1 °C / 24±3-44±4 h)
Bacillus subtilis ATCC 6633 according to ISO 4833 (30±1 °C / 72±3 h)
Escherichia coli ATCC 8739 according to ISO 16649 (44±1 °C / 21±3 h) / Escherichia coli ATCC 8739 according to ISO 21528 (37±1 °C / 24±2 h) / Escherichia coli ATCC 8739 according to ISO 4832 (30±1 °C / 24±2 h) / Escherichia coli ATCC 8739 according to ISO 4833 (30±1 °C / 72±3 h)
Escherichia coli O157 H7 ATCC 700728 according to ISO 16654 (37±1 °C / 21±3 h)
Listeria monocytogenes ATCC 13932 according to ISO 11290 (37±1 °C / 48±4 h)
Staphylococcus aureus ATCC 25923 according to ISO 6888 (37±1 °C / 24±2-48±2 h) / Staphylococcus aureus ATCC 25923 according to ISO 4833 (30±1 °C / 72±3 h)
Inoculation conditions: (100±20. Min. 50 CFU).
Reference media: Media batch TSA already validated.

According to ISO 11133 Water Microbiology:
Incubation conditions:
Escherichia coli ATCC 8739 according to ISO 9308 (36±2 °C / 21±3 h) / Escherichia coli ATCC 8739 according to ISO 6222 (36±2 °C / 44±4 h)
Escherichia coli ATCC 11775 according to ISO 9308 (36±2 °C / 20±2 h)
Clostridium perfringens ATCC 13124 according to ISO 14189 (44±1 ºC, anaerobic conditions / 21±3 h) / Clostridium perfringens ATCC 13124 according to ISO 6461 (37±1 ºC, anaerobic conditions / 44±4 h)
Pseudomonas aeruginosa ATCC 10145 according to ISO 16266 (44±4 h / 36±2 ºC)
Enterococcus faecalis ATCC 29212 according to ISO 7899 (36±2 ºC / 44±4 h).
Inoculation conditions: (100±20. Min. 50 CFU).
Reference media: Media batch TSA already validated.

Rest of strains; Streptococcus pneumoniae ATCC 6305, Streptococcus pyogenes ATCC 19615:
Incubation conditions: (30-35ºC / 18-24 h).
Reference media: Media batch TSA already validated.

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Good growth, >70%	
Candida albicans ATCC 10231	Good growth	
Escherichia coli ATCC 11775	Good growth, >70%	

Bacillus cereus ATCC 11778	Good growth, >70%	
Clostridium perfringens ATCC 13124	Good growth, >70%	
Listeria monocytogenes 4b ATCC 13932	Good growth, 70%	
Aspergillus brasiliensis ATCC 16404	Good growth	
Streptococcus pyogenes ATCC 19615	Good growth	Beta hemolysis
Staphylococcus aureus ATCC 25923	Good growth, >70%	Beta hemolysis
Enterococcus faecalis ATCC 29212	Good growth, >70%	
Streptococcus pneumoniae ATCC 6305	Good growth	Alpha hemolysis
Staphylococcus aureus ATCC 6538	Good growth	
Bacillus subtilis ATCC 6633	Good growth, >70%	
Escherichia coli O157:H7 ATCC 700728	Good growth, >70%	
Escherichia coli ATCC 8739	Good growth, >70%	
Pseudomonas aeruginosa ATCC 9027	Good growth	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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