BIOMÉRIEUX

REF 41466 / 41467

049325 - 01 - 2018-09

EN

Trypcase Soy Agar (TSA-F)

IVD

INTENDED USE

Isolation of non-fastidious microorganisms.

This medium is an isolation medium for the development of bacteria which do not have specific growth requirements.[1,2]

It can be used alone or supplemented with blood (sheep or horse blood).

Owing to the presence of blood, hemolysis can be revealed. This is a basic criterion for orienting bacterial identification.

It is used, in the pharmaceutical industry, for the enumeration of total aerobic microorganisms during the microbiological control of non-sterile products.

This medium complies with the performance requirements in the harmonized chapters of the European, United States, and Japanese Pharmacopoeias.[3, 4, 5]

EXPLANATION AND PRINCIPLE

It contains a mixture of peptones which encourage the growth of most microorganisms.

COMPOSITION OF THE MEDIUM

Theoretical formula

This medium has been adjusted and/or supplemented according to the performance criteria required:

Casein peptone (bovine)	15 g
Soy peptone	5 g
Sodium chloride	5 g
Agar	15 g
Purified water	1 L
pH 7.3	

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use and microbiological control.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- The media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents which show signs of contamination.
- Before use, make sure the tamper-proof systems are intact (capsule, seal, stopper).
- After regeneration, the entire contents of the bottle must be dispensed into plates (the medium cannot be liquefied several times).
- Interpretation of the test results must be made taking into consideration the patient's clinical history, the source of the specimen, macroscopic and microscopic aspects and, if necessary, the results of any other tests performed.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

- General microbiology laboratory equipment.
- Sterile Petri dishes.

- Water baths.
- Controlled atmosphere generators.
- Jars.
- Bacteriology incubator.

Or

- Thermoregulated chambers with a controlled atmosphere.

POSSIBLE ADDITIONAL REAGENTS

- Defibrinated sheep blood.
- Defibrinated horse blood.

STORAGE CONDITIONS

- The media can be stored in their box at +2°C/+25°C until the expiry date.
- After the unsupplemented medium has been dispensed into Petri dishes, it can be stored for 1 week at +2°C/+8°C.

SPECIMENS

For Use in Medical Microbiology:

All types of specimens may be used. They should be inoculated directly onto the agar.

Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

This medium can be used to subculture bacterial strains in order to obtain pure cultures.

For Use in Industrial Microbiology:

For the control of non-sterile pharmaceutical products, follow the recommendations in the harmonized chapters of the Pharmacopoeias for specimen preparation.

PROCEDURE

Preparation of Petri dishes

1. Loosen the cap on the bottle.
2. Place the bottle of agar in a water bath equipped with a security system set to approximately +50°C, increase the temperature to +95°C and leave the agar to melt (approximately 45 minutes).
3. Mix after screwing the cap back on (use protective gloves against thermal risks).
4. Leave the bottles between 1 and 5 minutes at room temperature on the lab bench before transferring them to a thermostatically controlled water bath set at +45°C/+50°C. Maintain the bottles at this temperature until use.
5. Dispense the bottles into Petri dishes (18–20 mL per plate).
6. After reconstitution and cooling of the medium, keep the plates at +2°C/+8°C.

Caution: Blood should be added after step 4, according to the procedure indicated in the corresponding reagent package insert.

Inoculation and incubation

For Use in Medical Microbiology:

1. **Allow reagents to come to room temperature.**
2. Inoculate the specimen or the strain to be tested.
3. Put the medium in a suitable atmosphere, if necessary using a controlled atmosphere generator.
4. Incubate the inverted plates at +37°C. The user is responsible for choosing the appropriate incubation temperature for the intended use, in accordance with current standards. Incubation time varies according to the type of specimen and the microorganisms being tested for.
5. Examine the cultures after 24-48 hours of incubation. In certain cases, it may be necessary to prolong incubation.

For Use in Industrial Microbiology:

For the control of non-sterile pharmaceutical products:

Refer to the method described in the harmonized chapters of the Pharmacopoeias.

For surface inoculation:

Allow reagents to come to room temperature.

For enumeration of total aerobic microorganisms in pharmaceutical or cosmetic products, Trypcase Soy agar dispensed into Petri dishes is inoculated on the surface and then incubated, for example for up to 5 days at +30°C/+35°C.

For other uses, follow the current reference method.

For inoculation by pour-plate:

As a general rule,

1. Transfer the sample volume into two empty Petri dishes.
2. Dispense approximately 15 mL of agar maintained at +45°C/+50°C.
3. Mix carefully.
4. Leave to set on a flat surface.
5. Incubate the inverted plates, for example up to 5 days at +30°C/+35°C. The user is responsible for choosing the appropriate incubation temperature for the intended use, in accordance with current standards.

RESULTS AND INTERPRETATION

- After incubation, observe the microbial growth.
- If the medium is supplemented with blood, record the presence of any characteristic hemolysis:
  - α hemolysis: greenish coloration around the colony.
  - β hemolysis: clear zone around or under the colony.
- Identification of the microorganisms isolated must be followed by appropriate additional tests.
- If pharmaceutical products are being tested, count the colonies obtained.

QUALITY CONTROL

For Use in Medical Microbiology:

Protocol:

The performance of the medium can be tested using the following strains:

- Unsupplemented medium: *Staphylococcus aureus* ATCC® 6538™.
- Medium with blood:
  - *Streptococcus pyogenes* ATCC® 19615™.
  - *Streptococcus pneumoniae* ATCC® 6305™ (incubation in a CO_2-enriched atmosphere).

Range of expected results:

Unsupplemented medium:

At +35°C ± 2°C, the tested strain should grow after 24 hours of incubation.

Medium with blood:

Strain	Results at +35°C ± 2°C	
Streptococcus pyogenes ATCC® 19615™	Growth after 24 hours	β hemolysis
Streptococcus pneumoniae ATCC® 6305™		α hemolysis

Note:

- It is the responsibility of the user to perform Quality Control taking into consideration the intended use of the medium, and in accordance with any applicable local regulations (frequency, number of strains, incubation temperature, etc.).
- If the medium is used for enumeration, Quality Control may be performed using a calibrated inoculum incubated at +30°C/+35°C for bacteria and +20°C/+25°C for yeasts and moulds.

For Use in Industrial Microbiology:

The control complies with the recommendations in the harmonized chapters of the Pharmacopoeias.[3,4,5]

LIMITATIONS OF THE METHOD

- Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.
- The type of hemolysis depends on the species in question and the specific behavior of each strain.

PERFORMANCE

Unsupplemented medium:

Performance was evaluated at +37°C using 16 bacterial strains (7 Gram-negative strains including 4 enterobacteria, 9 Gram-positive strains) and 1 yeast (*Candida*).

Nutrient capacity:

All the tested strains grew within 24 hours, with the exception of *Brucella*, *Neisseria* and *Corynebacterium* which showed growth within 48 hours.

Medium with blood (sheep and horse blood):

Performance was evaluated at +37°C using 11 bacterial strains (6 streptococci strains, 1 *Streptococcus pneumoniae* strain, 2 *Listeria* and 2 anaerobic strains).

Nutrient capacity:

All the tested strains grew within 24 hours.

Hemolysis:

α or β hemolysis characteristic of *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Listeria* was observed within 24-48 hours of incubation.

WASTE DISPOSAL

Unused reagents may be considered as non-hazardous waste and disposed of accordingly.

Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced, according to their nature and degree of hazardousness, and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. FINEGOLD S.M., MARTIN W.J., SCOTT E.G. – *Bailey and Scott's Diagnostic microbiology* – MOSBY – 1978.
2. MURRAY P.R., BARON E.J., PFALLER M.A. *et al.* – 1995 – *Manual of clinical microbiology, 6th ed.* – American Society for Microbiology, Washington, D.C. – ISBN 1-55581-086-1.
3. European Pharmacopoeia Ph. Eur. *.
4. United States Pharmacopoeia USP *.
5. Japanese Pharmacopoeia JP *.
6. Mac FADDIN J.F. – 1985 – *Media for isolation-cultivation-identification-maintenance of medical bacteria,* vol. 1, Williams and Wilkins, Baltimore.

* This document is in compliance with current version of Pharmacopoeias.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Date of manufacture

LIMITED WARRANTY

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

PACKAGING

Ready-to-use media

REF	Units/Pack	Short name
41466	6 x 200 mL bottles	TSA-F
41467	6 x 100 mL bottles	TSA-F

REVISION HISTORY

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2018-09	049325-01	Administrative	Formatting and wording changes. Updated sections: Intended Use / Reagents and Materials Required but not Provided / Warnings and Precautions / Storage Conditions / Procedure / Results and Interpretation / Waste Disposal / Literature References / Index of Symbols / Limited Warranty / Revision History

bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France

673 620 399 RCS LYON
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com