



TRYPTIC SOY BROTH WITH NaCl 10% AND SODIUM PYRUVATE

Dehydrated culture medium

1 - INTENDED USE

Selective broth the enumeration of *Staphylococcus aureus* in foods with MPN method.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	17.0 g
Soy peptone	3.0 g
Sodium chloride	95.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Sodium pyruvate	10.0 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

Staphylococcus aureus has been identified as the causative agent in many foods poisoning outbreaks. Processed foods may contain relatively small numbers of debilitated viable cells, whose presence must be demonstrated by appropriate methods.

Tryptic Soy Broth with NaCl 10% and sodium pyruvate is based on the ability of *S. aureus* to grow in presence of 10% NaCl.^{1,2}

This liquid selective medium is recommended by FDA BAM^{3,4} and AOAC⁵ for the enumeration of *S. aureus* by MPN method in products in which small numbers of cells of the target organism are expected to be sublethally injured or stressed by heating, freezing, or drying and in foods expected to contain large population of competing species. The formulation, with a concentration of 95 g/L of sodium chloride, meets the requirements of FDA-BAM.⁴

Essential growth factors are provided by tryptone and soy peptone. Sodium pyruvate aids in resuscitation of stressed cells. Glucose is a source of carbon and energy. Dipotassium hydrogen phosphate is used as buffering agent to control the pH in the medium. Sodium chloride at high concentration is the selective agent because it inhibits the growth of many competing bacteria without having an effect on staphylococcal growth.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 130 g in 1000 mL of cold purified water. Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute 10 mL into test tubes and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth with NaCl 10% and Sodium Pyruvate	Dehydrated medium	40215512	500 g (3.8 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, ancillary culture media and reagents.

8 - SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.^{3,5}

9 - TEST PROCEDURE

- Inoculate 3 tubes of Tryptic Soy Broth with NaCl 10% and Sodium Pyruvate with 1 mL portions of decimal dilutions of each sample.
- Incubate tubes 48 ± 2 hours at 35-37°C.
- Using 3 mm loop, transfer 1 loopful from each tube showing growth (turbidity) to plate of Baird-Parker Agar medium with properly dried surface. Vortex-mix tubes before streaking if growth is visible only on bottom or sides of tubes.
- Streak inoculum to obtain isolated colonies. Incubate plates 48 hours at 35-37°C.
- From each plate showing growth, transfer at least 1 colony suspected to be *S. aureus* into a small tube containing 0.2 – 0.3 mL of BHI Broth and emulsify thoroughly.
- After incubation at 35-37°C for 18-24 hours, perform coagulase test and ancillary identification tests (catalase test, anaerobic utilization of glucose and mannitol, lysostaphin sensitivity, thermostable nuclease production).³
- Report as *S. aureus*/g as MPN/g according to appropriate MPN tables.

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the liquid medium.

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
S. aureus ATCC 6538
E. coli ATCC 25922

INCUBATION T° / T / ATM
 37°C/24 H/A
 37°C/48 H/A

EXPECTED RESULTS
 growth
 inhibited

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 Broth with NaCl 10% and Sodium Pyruvate is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1.

Selectivity is assessed by dilution to extinction method with the following non-target strains: *E. faecalis* ATCC 19433, and *E. coli* ATCC 25922. After incubation at 37°C for 48 hours the growth of *E. faecalis* is partially inhibited while *E. coli* is totally inhibited.

13 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and 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 medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 products 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 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 the validation of their shelf life, according to the type (tubes/bottles) and the applied storage conditions (temperature and packaging). According to FDA-BAM⁴ the prepared medium may be stored up to 1 month at 4 ± 1°C.

15 – REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. Lancette GA, Bennet RW. Evaluation of an improved MPN medium for recovery of stressed and nonstressed *Staphylococcus aureus*. J Ass Off Chem 1986; 69:44-46
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 12: *Staphylococcus aureus*. Content current as of: 12/16/2019
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. M154a: Trypticase (Tryptic) Soy Broth with 10% NaCl and 1% Sodium Pyruvate. Content current as of: 10/24/2017
5. AOAC International. Official Methods of Analysis, AOAC International Arlington, VA. 16th ed., 1995 sec. 987.09.

TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 Store in a dry place	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/11

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

