

# STAA AGAR BASE STAA SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplement

## 1 - INTENDED USE

For the enumeration of *Brochothrix spp.* in food samples, especially meat and meat products.

## 2 – COMPOSITION\*

### STAA AGAR BASE

#### TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.00 g
Yeast extract	2.00 g
Dipotassium hydrogen phosphate	1.00 g
Magnesium sulphate	0.49 g <sup>^</sup>
Cycloheximide	0.05 g
Bromocresol purple	0.02 g
Agar	11.50 g

### STAA SELECTIVE SUPPLEMENT

#### (VIAL CONTENT FOR 500 mL OF MEDIUM)

Streptomycin sulphate	250 mg
Thallos acetate	25 mg

\*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

<sup>^</sup> corresponding to 1 g of magnesium sulphate heptahydrate

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Brochothrix* includes 2 species, *B. thermosphacta* and *B. campestris*.<sup>1</sup> *B. thermosphacta* is a Gram-positive, rod-shaped, nonmotile, non-spore-forming, facultative anaerobic, and psychrotrophic organism frequently involved in the spoilage of prepacked and vacuum-packaged meat or meat products.<sup>2</sup> *B. campestris* has so far only been identified in samples from soil and grass.<sup>1</sup> Streptomycin thallos acetate actidione (STAA) agar is based on the formulation described by Gardner<sup>2</sup> for the enumeration of *Brochothrix spp.* in foods, especially meat and meat products.

The complete medium is prepared with the basic medium to which glycerol and the selective supplement consisting of streptomycin and thallium acetate are added.

Peptone provides nitrogen and minerals for microbial growth; yeast extract is a source of B-vitamins complex for growth stimulation. Dipotassium phosphate is used as buffering agent to control the pH in the medium while magnesium sulphate enhances the microbial growth. Streptomycin sulphate suppressed the growth of some Gram-positive and most Gram-negative bacteria but has no effect on the development of *Brochothrix spp.* Thallos acetate inhibits most yeasts as well as many aerobic and facultatively anaerobic bacteria. Cycloheximide is included as antifungal agent.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 17.5 g in 500 mL of cold purified water and add 7.5 g of glycerol (REF 421015). Heat to boiling with frequent agitation to dissolve the medium completely and sterilize by autoclaving at 115°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of STAA Selective Supplement (REF 4240052), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

**WARNING:** the powder medium STAA Agar Base contains cycloheximide. Handle with care, avoiding contact with skin and eyes. The selective supplement contains thallos acetate. Don't inhale, avoid contact with skin and eyes. Handle the product wearing gloves and using eye protective gear. Read the Material Safety Data Sheets.

## 5 - PHYSICAL CHARACTERISTICS

### STAA Agar Base ISO

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid
Final pH at 20-25 °C	7.0 ± 0.1

### STAA Selective Supplement

Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid

## 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
STAA Agar Base	Dehydrated medium	4020792	500 g (14.5 L)
STAA Selective Supplement	Freeze-dried supplement	4240052	10 vials, each for 500 mL of medium

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, pipettes and spreaders, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, glycerol (REF 421015), ancillary culture media and reagents.

## 8 - SPECIMENS

Products intended for human consumption and animal feeding especially meat and meat products, environmental samples in the area of food and feed production, handling, and samples from the primary production stage. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.





### 9 - TEST PROCEDURE

1. Transfer, by means of a sterile pipette 0.1 mL of the test sample if the product is liquid, or of the initial suspension in the case of other products, to the STAA Agar plate.
2. Repeat the procedure using further decimal dilutions, if necessary.
3. Spread the inoculum as quickly as possible over the surface of the agar plate without touching the sides of the dish with the spreader. Leave the plates with the lids on for about 15 min at room temperature for the liquid to be absorbed into the agar.
4. Invert the prepared plates and incubate at 22°C / 25 °C for 48 h ± 4.

### 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

On STAA Agar, *Brochothrix* usually produce shiny, round or circular colonies of diameter 1 mm or larger with off-white colour.

Count the characteristic colonies on each dish containing 10 to 150 colonies.

Perform oxidase and catalase tests: *Brochothrix* is oxidase-negative and catalase positive.

### 11 - USER QUALITY CONTROL

All manufactured lots of the products 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	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>B. thermosphacta</i> ATCC 11509	22-25°C/ 44-52 H / A	good growth
<i>E. faecalis</i> ATCC 19433	22-25°C/ 44-52 H / A	totally or partially inhibited
<i>L. sakei</i> ATCC 15521	22-25°C/ 44-52 H / A	totally or partially inhibited

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

### 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated STAA Agar Base, supplemented with glycerol and STAA Selective Supplement, are tested for productivity and selectivity with target and non-target strains.

The productivity characteristics are tested by semi-quantitative ecometric technique with the target strain *B. thermosphacta* ATCC 11509. After incubation at 22°C for 48 hours the target strain exhibits good growth with pale yellow colonies.

Selectivity is assessed 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 following non-target strains: *E. faecalis* ATCC 19433, *L. sakei* ATCC 15521, *L. plantarum* ATCC 8014, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *C. albicans* ATCC 18804. The growth of the non-target strain is totally or partially inhibited after incubation at 22°C for 48 hours.

### 13 - LIMITATIONS OF THE METHOD

- *Pseudomonads* may grow on STAA medium. They shall be differentiated from *Brochothrix* spp. by performing the oxidase test.<sup>1</sup>
- Certain lactic acid bacteria may produce characteristic colonies on STAA medium. Lactic acid bacteria are differentiated from *Brochothrix* spp. by performing the catalase test.<sup>1</sup>

### 14 - PRECAUTIONS AND WARNINGS

- The products are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Thallium acetate has a high toxicity. Before use, consult the Material Safety Data Sheets.
- 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 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](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening the metal ring of the supplement vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website [www.biolifeitaliana.it](http://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

#### Dehydrated medium

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).

### Freeze-dried supplement














Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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 (plates/bottles) and the applied storage conditions (temperature and packaging). According to Baird RM *et al.* and ISO 13722, the self-prepared plates may be stored at between 2 °C and 8°C °C for up to 1 week and the bottled basal medium for 1 month at room temperature.1,3

### 16 - REFERENCES

1. ISO 13722:2017. Microbiology of the food chain — Enumeration of *Brochothrix* spp. — Colony-count technique.
2. Gardner GA. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. *J Appl Bacteriol.* 1966; 29:455-60.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. *Int J Food Microbiol* 1987; 5:270-1.

### TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature imitation	 Content sufficient for <n> tests	 Consult instructions for use	 Use by	 Keep away from direct light	 single-use

### REVISION HISTORY

Version	Description of changes	Date
Revision 1	Update of layout and content	2022/09

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

