STAPHYLOCOCCI 110 MEDIUM

Dehydrated culture medium

Staphylococci 110 Medium plate flooded with ammonium sulphate solution: gelatinase positive *S.aureus* colonies

1 - INTENDED USE

Selective medium for the isolation and differentiation of staphylococci.

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

Tryptone	10.0 g
Yeast extract	2.5 g
Gelatin	30.0 g
Lactose	2.0 g
Mannitol	10.0 g
Sodium chloride	75.0 g
Dipotassium hydrogen phosphate	5.0 g
Agar	15.0 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

Staphylococci 110 Medium, also known as Stone Gelatin Agar, is prepared on the basis of the formula described by Chapman[1] in 1946, following Stone's discovery in 1935[2] of the positivity to gelatinase test by food-poisoning staphylococci.
Staphylococci 110 Medium is a selective medium for the isolation and differentiation of staphylococci, based on the tolerance to high concentrations of sodium chloride, pigmentation of colonies, fermentation of mannitol and liquefaction of gelatin.[3] The medium complies with the formulations described by APHA[4] and AOAC[5].
The medium is suitable for isolation and differentiation of staphylococci for studies of food-poisoning outbreaks.[3]
Tryptone and yeast extract provide nitrogen, carbon, minerals and vitamins for microbial growth. Potassium phosphate prevents pH changes. The selectivity of the medium is due to the presence of a high NaCl content, which allows a good growth of staphylococci and a partial to total inhibition of Gram-negative bacteria and enterococci. Mannitol is included as a fermentable carbohydrate, lactose is an additional source of carbon; *S.aureus* ferments mannitol producing the acidification around the colonies; its fermentation can be detected by adding a few drops of bromocresol purple on the area of removed colonies from the agar plate, resulting in the production of a yellow colour. Gelatin serves as a substrate for gelatinase activity: gelatin hydrolysis is observed as clear zones around colonies after the addition of saturated aqueous solution of ammonium sulphate (Stone reaction).

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 149 g in 1000 ml of cold distilled water; heat to boiling with frequent agitation and autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	beige, hazy
Prepared plates appearance	beige, opalescent
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Staphylococci 110 Medium	Dehydrated medium	4020852	500 g (3.3 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Staphylococci 110 Medium is intended for the bacteriological processing of food samples. For samples collection and preparation, refer to the applicable international standards.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the sample 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.
Routinely incubate in aerobic atmosphere at 35°C for 43 hours. Alternatively incubate at 30°C for 48 hours. The incubation at 30°C produces a deeper pigmentation with no interference with Stone reaction or acid production from mannitol fermentation[3].

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
The presumptive differentiation between *S.aureus* and *S.epidermidis* is performed by reading the mannitol fermentation, gelatinase test and colony pigmentation.

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

S.aureus colony pigmentation: usually bright golden yellow-orange colour; *S.epidermidis, S.saprophyticus* colony pigmentation: white to cream coloured, basically non pigmented.
Mannitol fermentation: determine after reading colony pigmentation. Add a drop of 0,04% solution of bromocresol purple or bromothymol blue to the area where the colony was removed: a positive reaction results in an acid pH with resultant yellow colour.
Gelatinase activity: determine after recording mannitol fermentation. Flood the plate with 5 mL of saturated aqueous solution of ammonium sulphate pre-warmed at 35°C; incubate for 10 minutes at 35°C; a positive reaction is indicated by the formation of clear zones in an opaque white background, around pigmented colonies or areas of removed colonies.
Yellow-orange, gelatinase positive and mannitol positive colonies are presumptively identified as *S.aureus*.
White colonies, gelatinase positive and mannitol negative are probably *S.epidermidis*.
Confirm a possible *S.aureus* by testing for coagulase production that must be performed after the colony subculture in Nutrient Broth or BHI Broth or on a blood agar plate and incubation at 35°C for 18-24 hours; do not perform coagulase test directly with colonies grown on Staphylococci 110 Medium as salt content may interfere with coagulase results.
Emulsify 0.5 mL of broth culture or a colony from blood agar with 0.5 mL of rabbit plasma (Coagulase Plasma EDTA cat. no. 429937).
Incubate at 35-37°C and observe every 60 minutes in the first 4 hours of incubation for clotting by gently slanting the tube. Do not shake.
If no clot is observed by 4 hours, the tube should be read again after 18-24 h of incubation at 35-37°C.
This is because a small proportion of strains require longer than 4 hours for clot formation.

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	30-35°C / 44-48 H / A	growth, yellow colonies with a clear zone, mannitol positive
S. epidermidis ATCC 12228	30-35°C / 44-48 H / A	growth, white colonies with a clear zone, mannitol negative
E. coli ATCC 25922	30-35°C / 44-48 H / A	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 Staphylococci 110 Medium is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 30-35°C for 44-48 hours, with 2 target strains ATCC derivatives (*S.aureus* ATCC 25923, *S.aureus* ATCC 6538) and 1 *S.aureus* strain, isolated from food. After incubation the target strains show a good growth with typical yellow colonies surrounded by a clear zone and positive to mannitol fermentation test.
Specificity is evaluated by semi-quantitative ecometric technique, by incubating at 30-35°C for 44-48 hours, with one coagulase negative strain: *S.epidermidis* ATCC 12228. After incubation the strain show a good growth with white colonies surrounded by a clear zone and negative to mannitol fermentation test.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target strains *E.faecalis* ATCC 19433, *E.coli* ATCC 25922 and *P.vulgaris* ATCC 9484. The growth of non-target Gram positive strains is inhibited at the dilution 10^{-1}.

13 - LIMITATIONS OF THE METHOD

- *E.faecalis* and other enterococci may exhibit growth and slight mannitol fermentation; however the colonies are tiny and are easily differentiated from staphylococci by Gram staining and catalase test (*E.faecalis*: catalase negative, cocci in chains; staphylococci: catalase positive, cocci in clusters).[3]
  On primary isolation from the sample *S.aureus* can produce a bright golden yellow-orange pigmentation of the colonies; however, they may also be white or even colorless. The optimal temperature for the production of pigments is slightly lower (25-30°C) than that required for growth. However, colony pigmentation is not a reliable criterion for the differentiation of species of the genus *Staphylococcus*.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product 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 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 shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Chapman, G.H. (1946). J. Bacteriol. 51:409.
2. Stone R. V. (1935) Proc. Soc. Exper. Biol. & Med. 33. 185-187.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. American Public Health Association (1978) Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.
5. Association of Official Analytical Chemists (1992) Bacteriological Analytical Manual. 7th Edn. AOAC. Washington DC.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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	2020/05
Revision 2	Update of “intended use”, “test procedure”, “precautions and warnings” and “storage conditions and shelf life”	2022/05

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