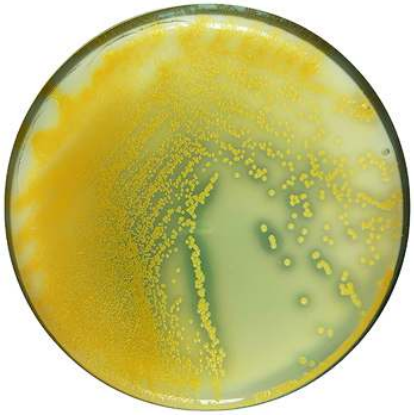
CHAPMAN STONE MEDIUM

Dehydrated culture medium



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 g
Yeast Extract	2 g
Gelatin	30 g
D-Mannitol	10 g
Sodium Chloride	55 g
Ammonium Sulphate	75 g
Dipotassium Hydrogen Phosphate	5 g
Agar	15 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

Chapman Stone Medium is prepared on the basis of the formula described by Chapman[1] in 1948, which represents a modification of the Chapman's Staphylococcus Medium described in 1946[2], in which sodium chloride concentration is reduced to 5.5% and the ammonium sulphate is incorporated into the medium rather than applied to the plate after incubation. The medium was developed by Chapman, following Stone's observations of 1935[3] on the positivity to gelatinase test by staphylococci.
Chapman Stone Medium is a selective medium for the isolation and differentiation of staphylococci, based on the tolerance to high concentrations of sodium chloride, fermentation of mannitol and liquefaction of gelatin.
The medium is suitable for isolation and differentiation of staphylococci and for studies of food-poisoning outbreaks.[4] Chapman Stone Medium is included by Atlas in the reviews of culture media for isolation and differentiation of staphylococci from food[5] and environmental samples[6].
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 relatively 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: *Staphylococcus aureus* ferments mannitol producing the acidification around the colony; its fermentation can be detected by adding a few drops of bromo-cresol 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.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 202 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	white, fine, homogeneous, free-flowing powder
Solution appearance	yellowish, hazy
Prepared plates appearance	yellowish, opaque
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chapman Stone Medium	Dehydrated medium	4013002	500 g (2.5 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

For food samples, refer to the applicable international standards.
Good laboratory practices for collection, transport and storage of specimens should be applied.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen 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.
Incubate inoculated plates in aerobic atmosphere at 30-35°C for 44-48 hours.

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 and gelatinase tests.

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Mannitol fermentation: add 1 drop of 0,04% solution of bromocresol purple to the area where the colony has been removed: positive results are indicated by yellow colour (acid production).
Gelatinase activity: the inoculated medium is opaque and whitish in colour, owing to precipitates that form between the gelatin and ammonium sulphate; a positive reaction is indicated by a clear zone around the colony.
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 30-35°C for 18-24 hours; do not perform coagulase test directly with colonies grown on Chapman Stone Medium as salt content ~~m~~ 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 examine hourly up to 3-6 hours. Do not shake or agitate the tube. Gently slant and examine for a clot which gels the whole contents of the tube or forms a loose web of fibrin.
If negative by the end of 3-6 hours, incubate overnight and re-examine at 24 hours. 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, colonies with a clear zone, mannitol positive
S.epidermidis ATCC 12228	30-35°C / 44-48 H / A	growth, colonies with a clear zone, mannitol negative
Escherichia 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 Chapman Stone 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 2 *S.aureus* strains, isolated from clinical specimens. After incubation the target strains show a good growth with typical 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 strains: *S.epidermidis* ATCC 12228. After incubation the strain show a good growth with 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).
- 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 GH. An Improved Stone medium for the isolation and testing of food-poisoning Staphylococci. Food Res 1948; 13:100.
2. Chapman GH. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation, and the Stone reaction. J Bacteriol 1946; 51:409-410.
3. Stone RV. A cultural method for classifying Staphylococci as of the “food poisoning” type. Proc Soc Exper Biol Med 1935; 33:185-187.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Atlas R. The Handbook of Microbiological Media for the Examination of Food. CRC Press,1995
6. Atlas R. The Handbook of Microbiological Media for Environmental Microbiology. CRC Press,1995

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”, “limitation of the method”, “precautions and warnings”	2022/05

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

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