VOGEL JOHNSON AGAR

Dehydrated culture medium

S.aureus on
Vogel Johnson Agar

1 - INTENDED USE

Selective medium for the isolation and differentiation of *Staphylococcus aureus.*

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

Tryptone	10.000 g
Yeast extract	5.000 g
Mannitol	10.000 g
Dipotassium hydrogen phosphate	5.000 g
Lithium chloride	5.000 g
Glycine	10.000 g
Phenol red	0.025 g
Agar	16.000 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

Vogel Johnson Agar is prepared on the basis of the formula described by Vogel and Johnson[1] in 1960, as a modification of Tellurite Glycine Agar of Zebovitz, Evans and Niven[2] of 1955. Vogel and Johnson increased the mannitol content and added phenol red as a pH indicator.
Vogel Johnson Agar is a selective medium for the isolation and differentiation of *S.aureus.* Its use has been described for clinical specimens[3] for the examination of cosmetics[4,5], pharmaceutical products[6], swimming pool water[7], milk[8] and, supplemented with oxacillin, for the detection of methicillin resistant staphylococci, from clinical samples[9].
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 lithium chloride and glycine and to the addition of potassium tellurite, which allows a good growth of staphylococci and the inhibition of almost all normal upper respiratory tract flora, mainly within 24 hours of incubation.[3] Mannitol is included as a fermentable carbohydrate: *S.aureus* ferments mannitol producing the acidification around the colony, resulting in a yellow colour change of medium; potassium tellurite forms a black precipitate inside the colonies when reduced by staphylococci to metallic free tellurium.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 61 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 20 mL of Potassium Tellurite 1% Solution (REF 42211501). A less selective medium may be prepared by adding 10 mL/L of 1% Potassium Tellurite Solution. Do not heat medium after addition of potassium tellurite.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Vogel Johnson Agar	Dehydrated medium	4021922	500 g (8,2 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. Potassium Tellurite 1% Solution (REF 42211501).

8 - SPECIMENS

Vogel Johnson Agar is intended for the bacteriological processing of food, pharmaceutical, cosmetic and environmental samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. For non clinical samples, refer to the applicable international standards.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Spread from 0.1 to 1.0 mL of diluted sample suspended in 0.1% Peptone Water over the surface of the well dried plate. Incubate at 35-37°C and examine after 24 and 48 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
S.aureus grows with large black convex shiny colonies, surrounded by a yellow halo.
Coagulase-negative staphylococci grow poorly with black colonies, without yellow zone; the medium surrounding colonies may be a deeper red colour due to utilisation of peptones with resultant alkalinity.

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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 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	35-37°C / 18-24 H / A	growth, black colonies with a yellow halo
S.epidermidis ATCC 12228	35-37°C / 18-24 H / A	slight growth, black colonies
E.coli ATCC 25922	35-37°C / 18-24 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 Vogel Johnson Agar 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 35-37°C for 18-24 hours, with one target ATCC derivative strain (*S.aureus* ATCC 25923) and two *S.aureus* strains, isolated from food. After incubation the target strains show a good growth with typical black colonies surrounded by a yellow zone.
Specificity is evaluated by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with one coagulase negative strain: *S.epidermidis* ATCC 12228. After incubation the strain shows a slight growth with black colonies surrounded by a deep red zone.
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 *E.coli* and *E.faecalis* is inhibited at the dilution 10^{-1}, the growth of *P.vulgaris* is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- During the first 24 hours of incubation most organisms other than coagulase-positive staphylococci are inhibited; however after this incubation period other organisms may exhibit slight growth, especially enterococci and *S.epidermidis.*
- *Proteus* spp. after 18 hours grow with black colonies and with a change in the colour of the medium to brown: however, after 48 hours of incubation there is an alkaline inversion of the pH with the development of a purple colour.
- 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. If relevant, perform antimicrobial susceptibility testing.

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. Vogel TA, Johnson M. A modification of the Tellurite-Glycine Medium for use in the identification of Staphylococcus aureus. Public Health Lab. 1960; 18:131.
2. Zebovitz E, Evans JB, Niven Jr CF. Tellurite-Glycine Agar; a selective plating medium for the quantitative detection of coagulase-positive staphylococci. J Bacteriol 1955; 70:686.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. U.S. Food & Drug Administration.BAM: Bacteriological Analytical Manual, Chapter 23: Microbiological Methods for Cosmetics 10/31/2017
5. Curry, Graf and McEwen (ed.). 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.

6. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
7. Alico RK, Dragonjact MF. Evaluation of culture media for recovery of Staphylococcus aureus from swimming pools. App Environ Microbiol 1986; 51:699-702.
8. Halpin-Dohnalek MI, Marth EH. Growth of Staphylococcus aureus in milks and creams with various amounts of milk fat. J Food Prot 1989; 52:540-543
9. Flournoy DJ, Wongpradit S, Silberg SL. Screening media for detection of Methicillin-Resistant Staphylococcus aureus from non-sterile body sites. Med Microbiol Immunol 1990; 179:25-30

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.

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