

VERA CTSA MEDIUM

Dehydrated culture medium

1 - INTENDED USE

General purpose medium base for fermentation reactions of microorganisms, for motility test and for the maintenance of microbial strains.

2- COMPOSITION

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

Tryptone	20.000 g
L-Cystine	0.500 g
Sodium chloride	5.000 g
Sodium sulphite	0.500 g
Phenol red	0.017 g
Agar	2.500 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

Vera Cystine Tryptic Semisolid Agar (CTSA) is a semi-solid medium used for the growth and maintenance of pathogenic microorganisms^{1,2} and for motility tests³. When supplemented with carbohydrates, it is used to study the fermentative metabolism of bacteria.^{3,4}

Tryptone provides nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance. Vera⁴ who developed the medium reported that sodium sulphite and L-cysteine enhance the growth of *N.gonorrhoeae*. Phenol red is a pH indicator: when the medium is prepared with a final concentration of 0.5-1 % carbohydrate, most of the end products of its fermentation are organic acids, which produce a colour change of the pH indicator from red to yellow; If the test is negative, a catabolic attack of peptones will occur with the formation of ammonia, the alkalisation of the medium and a colour change of phenol red from red-orange to reddish-pink. The 0.25% agar concentration reduces the diffusion of O₂ into the medium and the diffusion of the CO₂ produced. Therefore, the growth of anaerobic microorganisms is permitted. Moreover, this low concentration of agar makes the medium particularly suitable for use in motility tests.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and autoclave at 118 °C for 15 minutes. To prepare fermentation medium, add 5-10 g of carbohydrate before autoclaving or dissolve medium in 900 mL water, autoclave, and aseptically add 100 mL sterile 5-10% carbohydrate solution.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red-orange, limpid
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Vera CTSA Medium	Dehydrated medium	4021832	500 g (17.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Pure cultures from general-purpose solid media (e.g., chocolate agar).

9 - TEST PROCEDURE

With fresh colony growth prepare a heavy inoculum emulsified in 0.5 mL sterile saline.

With sterile pipet add 2-3 drops of test organism suspension onto surface of medium.

With plugged sterile capillary pipet stab upper 1/3 of medium several times.

Incubate aerobically or anaerobically depending upon the organisms being tested at 35-37°C for 48-78 hours

Examine daily for growth (turbidity), evidence of motility and acid production.

10 - READING AND INTERPRETATION

Positive test, acid production: yellow colour first appearing at surface and progressing usually only in area of stab

Negative test: red (alkaline) to orange (neutral) colour.

Compare with uninoculated tube.

Motile organisms show growth out from the line of stab inoculation. Nonmotile organisms only grow along the stab line.

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.

CTSA with glucose: *N.gonorrhoeae* ATCC 9013: growth, the medium turns to yellow after incubation at 35-37°C for 24 hours

CTSA with glucose: *B.catharralis* ATCC 8176: growth, the medium does not turn to yellow after incubation at 35-37°C for 24 hours

ATCC is a trademark of American Type Culture Collection





12 - LIMITATIONS OF THE METHOD

- For maximum efficiency, the medium should be used freshly prepared or boiled, with caps loosened, and cooled just before inoculation.³
- Addition of more than 0.5% carbohydrates may necessitate adjustment of pH.³
- CTSA requires heavy inoculum.³
- N.gonorrhoeae* may grow poorly and with atypical patterns due to their fastidiousness.³
- Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with some *Neisseria* pathogens.³
- Neisseria* spp. usually produce acid only in the area of stabs (upper third); if there is a strong yellow colour throughout the medium, it is a possible contaminant. If in doubt about a tube containing a *Neisseria* spp. a Gram stain and oxidase test should be performed on the growth: *Neisseria* spp. are Gram-negative kidney shaped diplococci, oxidase positive.³
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

13 - PRECAUTIONS AND WARNINGS

- This product is for Laboratory use 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.

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 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).

15 - REFERENCES

- Alford JA, Wiese EE, Gunter JJ. Heat resistance in *Corynebacterium* and the relationship of the genus to *Microbacterium*. *J Bacteriol* 1955; 69:516
- Myers RM, Koshy G. Beta-haemolytic streptococci in survey throat cultures in an Indian population. *Am J Public Health* 1961.; 52:1872
- MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
- Vera HD. A simple medium for identification and maintenance of the gonococcus and other bacteria. *J. Bacteriol* 1948; 55:531-536

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 2	Updated layout and content	2022/05

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

