

CHOCOLATE AGAR ENRICHED

Ready-to-use plates



Neisseria gonorrhoeae
on Chocolate Agar Enriched

1 - INTENDED USE

In vitro diagnostic device. Non selective, general-purpose medium for the isolation and cultivation of nutritionally fastidious microorganisms, from clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

Peptocomplex	15 g
Corn starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g
80°C heated defibrinated sheep blood	50 mL
Purified water	1000 mL
Biovitex Enrichment Supplement	
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Coccarboxylase	1 mg
p-aminobenzoic acid	0.13 mg
Thiamine	0.03 mg
Vitamin B12	0.1 mg
L-glutamine	100 mg
L-cystine	11 mg
L-cysteine HCl	259 mg
Adenine	10 mg
Guanine HCl	0.3 mg
Ferric nitrate.6H ₂ O	0.2 mg
Glucose	1 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

In 1945, Johnston¹ described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 hours as opposed to previous 48 hours methods. This medium was later modified by Carpenter and Morton² using GC Medium Base with the addition of haemoglobin and a yeast concentrate. The medium was further improved by replacing yeast concentrate with a chemically defined supplement formulated specifically to facilitate the growth of gonococci.³

Chocolate Agar Enriched is a non-selective, general-purpose medium, prepared with GC Medium Base, supplemented with heated defibrinated horse blood and Biovitex, for the isolation and cultivation of nutritionally fastidious microorganisms from clinical specimens.^{4,5} Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevents pH changes due to amine production; corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.⁴ Heated horse blood provides hemin (X factor) required for growth of *Haemophilus* and enhances growth of *Neisseria*. The medium is supplemented with Biovitex that provides V factor (NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	brown, opaque
Final pH at 20-25 °C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chocolate Agar Enriched	Ready-to-use plates	541521	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, CO₂ generators and jars or CO₂ incubator with humidifier, ancillary culture media and reagents for colony identification.

7 - SPECIMENS

Chocolate Agar Enriched plates can be directly inoculated with many clinical specimens collected from various normally non-sterile human sites **such as, throat, genital, nose, wounds and from primarily sterile body sites**. Refer to the quoted literature for specimens' types, related to specific infections.^{6-8,10-12} Chocolate Agar Enriched is not suitable for direct inoculation of blood samples **but may be used as subculture medium from blood cultures**. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.^{6,8}





8- TEST PROCEDURE

Allow plates to come to room temperature. 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 at 35-37°C in aerobic conditions with 5 -10% CO₂, and record the results after 18-24 and 48 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols. Consult the procedures outlined in the references for further information.^{7,8, 10, 11}

9 - READING AND INTERPRETATION

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

Colonies of *Haemophilus influenzae* have a diameter of about 1-2 mm, are colourless, transparent, moist and tend to be translucent, with a characteristic "mousy" odour.

Colonies of *N. gonorrhoeae* are of variable diameter (0.5 - 2 mm), moderately convex, raised, finely granular, glistening, with entire or lobate margins.

For other fastidious microorganisms, refer to appropriate references and procedures for results reading and interpretation.^{7,8,10,11}

10 - 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 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>H. influenzae</i> ATCC 10221	35-37°C / 18-24H / CO ₂	good growth
<i>N. gonorrhoeae</i> ATCC 43069	35-37°C / 18-24H / CO ₂	good growth

ATCC is a trademark of American Type Culture Collection

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to use plates of Chocolate Agar Enriched and of the raw material used for the production of prepared plates (dehydrated GC Medium Base REF 401520 supplemented with Biovitex and heated defibrinated horse blood) are tested for productivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative ecometric technique with the following strains: *H. influenzae* ATCC 10221, *N. gonorrhoeae* ATCC 43069, *S. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 6305, *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433. After incubation at 35-37°C for 18-24 hours the amount of growth is evaluated and recorded. All strains show a good growth in both batches.

Accuracy was assessed by reviewing the Quality Control data. The results of 27 batches produced from 9/8/2022 to 16/9/2025 were evaluated. Correct detection of fastidious microorganisms is confirmed by the inclusion of well-characterised strains in the QC process. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity with tested strains.

12 - LIMITATIONS OF THE METHOD

- The presence of small particles may sometimes be observed in the agar. However, this phenomenon does not affect the performance of the medium.
- The growth on Chocolate Agar Enriched depends on the metabolic requirements of each microorganism; some target strains may not be able to grow or may show a delayed growth. A lack of growth does not preclude the presence of infectious agents in the sample.
- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional selective media such as Thayer Martin for the isolation for gonococcus and chocolate agar with bacitracin for the isolation of *H. influenzae*.
- For the growth of *N. gonorrhoeae*, it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile distilled water. Place damp gauze or paper towels in the CO₂ container before incubation or use an incubator with humidifier.⁴
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N. gonorrhoeae*.⁴
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N. gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.⁴
- If *N. gonorrhoeae* is suspected, the incubator temperature should be set at 35-36,5°C with 5% CO₂, because many strains of *N. gonorrhoeae* will not grow well at 37°C and grow poorly with 10% CO₂.^{4,9}
- The device is not intended to diagnose infections or to guide the antimicrobial therapy. It is used in a diagnostic set of investigations to provide microbial colonies isolated from clinical samples of patients with suspected microbial infection.
- The presence of colonies on Chocolate Agar Enriched is not an indication, by itself, of the presence of pathogenic microorganisms: user must differentiate potential pathogens requiring biochemical, immunological, molecular, or mass spectrometry testing for identification and antimicrobial testing from contaminants that represent members of normal microbiota.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic device intended for professional use only, is not automated and is not a companion diagnostic tool. It must be used by adequately trained and qualified laboratory personnel, observing biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. Therefore, it is recommended that the ready-to use plates 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 for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.





- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal and dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains, in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify the Manufacturer (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The Manufacturer may not be held responsible for any loss or damage in any way resulting from or related to use of the product in manners not compliant with the instructions provided.















14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Johnston J. Comparison of gonococcus cultures read at 24 and 48 hours. *J Vener Dis Inform* 1945; 26:239.
2. Carpenter CM, Morton HE. An improved medium for isolation of the gonococcus in 24 hours. *Proc. N.Y. State Assoc. Public Health Labs* 1947; 27:58-60.
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4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Atlas R, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
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7. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
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9. CDC: Morbidity and Mortality Weekly Report (MMWR).Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15
10. UK Standards for Microbiology Investigations, UKHSA, ID. 'Identification of Neisseria Species.ID, Issue number: 4.1, Issue date: 11.07.25 <https://www.gov.uk/government/publications/smi-id-6-identification-of-neisseria-species>.
11. UK Standards for Microbiology Investigations, UKHSA, ID.. 'Identification of Haemophilus species and the HACEK group of organisms'. ID 12, Issue number: 5.1, Issue date: 17.07.25. <https://www.gov.uk/government/publications/smi-id-12-identification-of-haemophilus-species-and-the-hacek-group-of-organisms>.
12. UK Standards for Microbiology Investigations. Investigation of bronchoalveolar lavage, sputum and associated specimens. B 57, Issue no: 3.5, Issue date: 08.05.19 <https://www.gov.uk/government/publications/smi-b-57-investigation-of-bronchoalveolar-lavage-sputum-and-associated-specimens>

TABLE OF APPLICABLE SYMBOLS

 Catalogue number	 Batch code	 <i>In vitro</i> diagnostic medical device	 Manufacturer	 This way up	 For single use only	 European conformity mark
 Temperature limitations	 Contents sufficient for <n> tests	 Consult electronic instructions for use	 Use by	 Keep away from sunlight	 Fragile, handle with care	 Unique device identifier

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03
Revision 3	Limitations of the method, precautions and warnings, references, table of applicable symbols.	2025/11

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

