INSTRUCTIONS FOR USE

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
Cocarboxylase	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.$6H_20$	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, Johnston[1] 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 Morton[2] 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, CO_2 generators and jars or CO_2 incubator with humidifier, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Chocolate Agar Enriched plates can be directly inoculated with many clinical specimens collected from various normally sterile and non sterile human sites. Refer to the quoted literature for specimens types, related to specific infections.[6-8] Chocolate Agar Enriched is not suitable for direct inoculation of blood samples. 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]

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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% CO_2, 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]

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" odor.
Colonies of *N.gonorhoeae* 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 - 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
H.influenzae ATCC 10221	35-37°C / 18-24H / CO_2	good growth
N.gonorrhoeae 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.

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; it is possible that some strains are unable to grow on the medium.
- Depending on the specimens analyzed 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 CO_2 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% CO_2, because many strains of *N. gonorrhoea*e will not grow well at 37°C and grow poorly with 10% CO_2.[4,9]
- 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.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved 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. 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 the product doesn't contain any transmissible pathogen. 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 by Biolife Italiana 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 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 are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the in vitro diagnostic.
- 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 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.
3. Martin JE Jr, Billings TE, Hackney JF, Thayer JD. Primary isolation of N.gonorrhoeae with a new commercial medium. Public Health Rep. 1967; 82:361-363.
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.
6. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
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.
8. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019.
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

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03

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

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