

AG-CHOCOLATE AGAR - INSTRUCTIONS FOR USE

(Ready Plated Media)

1. INTENDED USE:

An in vitro diagnostic general-purpose medium, used with specific enrichments and selective supplements, for isolating and cultivating *Neisseria gonorrhoeae*, *Haemophilus spp.*, and other fastidious microorganisms from clinical specimens.

2. PRINCIPLE:



Chocolate Agar: Colonies of *N.gonorrhoeae*

In 1945, Johnston 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 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. Chocolate Agar Enriched is a non-selective, general-purpose medium, prepared with GC Medium Base, supplemented with heated defibrinated horse blood for the isolation and cultivation of nutritionally fastidious microorganisms from clinical specimens. Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevent 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. Heated horse blood provides hemin (X factor) required for growth of *Haemophilus* and enhances growth of *Neisseria*.

MATERIALS PROVIDED:

PRODUCT	TYPE	REF	PACK
AG - Chocolate Agar plate	Ready Plated Media	AG/CA/22/01	10 plates in a pack

3. MATERIALS REQUIRED BUT NOT PROVIDED:

Sterile loops and swabs, incubator and laboratory equipment as required, CO₂ generators and jars or CO₂ incubators with humidifiers, ancillary culture media and reagents for the identification of the colonies.

4. SPECIMENS:

Chocolate Agar Enriched plates can be directly inoculated with a variety of clinical specimens obtained from both normally sterile and non-sterile human sites. Refer to the quoted literature for specimen 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.

5. TEST PROCEDURE, READING AND INTERPRETATION:

- 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.

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- 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 colorless, transparent, moist and tend to be translucent, with a characteristic “mousy” odor.
- 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.

6. USER QUALITY CONTROL :

All manufactured lots of the products are released for sale after 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 quality control.

CONTROL STRAINS	INCUBATION	EXPECTED RESULTS
<i>H.influenzae</i> ATCC 10221	35-37°C /18-24H – 5%CO ₂	Good growth
<i>N.gonorrhoeae</i> ATCC 43069	35-37°C /18-24H – 5%CO ₂	Good growth

Key: ATCC is a trademark of American Type Culture Collection

7. LIMITATIONS OF THE METHOD:

- 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
- Addition of Horse blood or rabbit blood to base medium supports growth of *H. haemolyticus* but resemble beta hemolytic Streptococci and hence must be confirmed.
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N.gonorrhoeae*.
- 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.
- The presence of colonies on Chocolate Agar Enriched is not an indication, by itself, of the presence of pathogenic microorganisms: users 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.

8. 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.
- 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 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 with AstraGene and can be provided on request.

9. STORAGE CONDITIONS AND SHELF LIFE:

- Upon receipt, store at +2 - 8°C away from direct light in a cool, dry place. Storage below 2°C may lead to crystallization of media components.
- If properly stored, it may be used up to the expiration date. Do not use it beyond this date. The user is responsible

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for the storage method (temperature) of the medium.

- For optimal usage, it is advisable to utilize the entire pack of 10 plates once they have been taken out of the packaging.

10. SYMBOLS:



Date of manufacture



Use-by-date



Do not use if package is damaged



Manufacturer



Batch Code



Refer to the instructions



ISO



GMP



In-Vitro diagnostic Medical devices



Mark of conformity



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