

CHOCOLATE AGAR BACITRACIN

Ready-to-use plates



Chocolate Agar Bacitracin:
colonies of *Haemophilus influenzae*

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of *Haemophilus* spp. from clinical specimens with mixed flora.

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 horse blood	70 mL
Bacitracin	20.000 UI
Vancomycin	5 mg
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

The genus *Haemophilus* includes clinically relevant Gram-negative coccobacilli that grow optimally at 35–37 °C. *Haemophilus influenzae* is a key pathogen associated primarily with upper respiratory tract infections.

In 1969 Hovig and Aandahl¹ formulated a selective medium for the isolation of *Haemophilus* spp. from respiratory tract, incorporating bacitracin 300 mg/L into chocolate agar. The use of the selective medium increased the isolation rate of *Haemophilus* sp. from all specimens: for nose swabs the isolation rate increased from 32% to 41.3%, for throat swabs from 30.7% to 98.7%, for sputum samples from 3.4% to 61.4%.¹

Chocolate Agar Bacitracin is a selective medium prepared with GC Medium base, supplemented with heated defibrinated horse blood, Biovitex, bacitracin and vancomycin, for the isolation and cultivation of *Haemophilus* spp. from clinical specimens contaminated by less fastidious commensal bacteria.²⁻⁴ 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* spp. The medium is supplemented with Biovitex that provides V factor (NAD) vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors which improve the growth of species requiring V factor for the growth: *H. influenzae*, *H. ducrey*, *H. aegyptius*, *H. haemolyticus*.² Bacitracin suppresses the growth of most strains of streptococci, staphylococci, micrococci and *Neisseria*, vancomycin is active against Gram positive bacteria.

4 - PHYSICAL CHARACTERISTICS

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

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chocolate Agar Bacitracin	Ready-to-use plates	541519	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, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Chocolate Agar Bacitracin plates can be directly inoculated with physiologically non-sterile clinical specimens collected from human sites such as ear and respiratory tract.²⁻⁴ Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.³⁻⁵





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 a moist atmosphere in the presence of 5-10% CO₂ and record the results after 24 and 44-48 hours, to obtain satisfactory growth of *H. influenzae* and most other *Haemophilus* species. When specimens for *H. aegyptius* and *H. ducreyi* are cultured, incubation may be necessary for up to 5 days.² Further, when *H. ducreyi* is suspected in the specimen, plates should be incubated at 30-33°C in 5% CO₂ in a high-moisture environment.²

9 - READING AND INTERPRETATION

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

The colonies' morphology of *Haemophilus* spp. are summarized here below.²

Colonies of *H. influenzae* have a diameter of about 1-2 mm, are smooth, low, convex, greyish, and translucent, with a characteristic "mousy" odour (non-indole producing strains) or a strong amine-like odour (indole producing strains)

Colonies of *H. aegyptius* reach a colony size of 0.5 mm after 48 hours of incubation; colonies are low, convex, translucent with a smooth entire surface.

Colonies of *H. parainfluenzae* are typically off-white to yellow and, like *H. influenzae*, 1 to 2 mm in diameter. The colony appearance is extremely varied.

Colonies of *H. haemolyticus* are translucent, smooth, and convex.

Colonies of *H. ducreyi* are small, flat, grey, and smooth.

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 10211	35-37°C / 24-48H / CO ₂	good growth
<i>S. pyogenes</i> ATCC 19615	35-37°C / 44-48H / CO ₂	growth inhibited

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 Bacitracin are tested for productivity and selectivity.

Productivity is tested by semi-quantitative ecometric technique with *H. influenzae* ATCC 10211 and *H. influenzae* ATCC 49247. After incubation at 35-37°C for 18-24 hours, with 5-10% of CO₂ the amount of growth is evaluated and recorded. The target strains show a good growth.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *S. pyogenes* ATCC 19615 and *S. aureus* ATCC 25923. After incubation at 35-37°C for 44-48 hours, with 5-10% of CO₂, the growth of non-target strains is totally inhibited.

Accuracy was assessed by reviewing the Quality Control data. The results of 30 batches produced from 09/11/2017 to 20/04/2020 were evaluated. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity with target strains and selectivity with non-target 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.
- *E. coli*, some *Neisseria* and *Candida* species, *Klebsiella*, *Proteus*, and *Pseudomonas* spp., as well as other Gram-negative bacteria may grow on this medium.
- The device is not intended to diagnose *Haemophilus* 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 *Haemophilus* infection
- Growth on the medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow or may show a delayed growth. A lack of growth or the absence of typical colonies does not preclude the presence of *Haemophilus* in the sample.
- A single medium is only rarely useful to recover the target-strains contained in a specimen, so concomitant cultures are necessary to recover organisms for typing.
- Appropriate tests are required for complete identification and epidemiological typing of colonies; if necessary, perform antimicrobial susceptibility tests using recommended methods.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative in vitro diagnostic (IVD) device intended for professional use only. It is not automated and is not a companion diagnostic. It shall be used by appropriately trained and qualified laboratory personnel, applying standard biohazard precautions and aseptic techniques.
- This product is not classified as hazardous under current European legislation.
- This culture medium contains raw materials of animal origin. Therefore, the ready-to-use plates should be handled as potentially infectious and used with appropriate precautions: do not ingest or inhale and avoid contact with skin, eyes, and mucous membranes. The TSE Statement describing the risk-reduction measures related to transmissible spongiform encephalopathies is available from the Manufacturer's website (www.biolifeitaliana.it).
- All laboratory specimens shall be considered potentially infectious.
- The laboratory area shall be controlled to prevent contamination by culture media and/or microbial agents.
- Each plate is for single use only.





- Ready-to-use plates are not sterile products, as they are not terminally sterilized; they are manufactured with controlled bioburden within the limits defined in the specifications reported on the Quality Control Certificate.
- Decontaminate and sterilize all biohazardous waste prior to disposal. Dispose of unused medium and plates inoculated with clinical samples or microbial strains in accordance with applicable local regulations.
- The Certificates of Analysis and the Safety Data Sheet are available on the Manufacturer's website (www.biolifeitaliana.it).
- Notify the Manufacturer (complaint@biolifeitaliana.it) and the relevant competent authorities of any serious incident occurring in connection with the use of this IVD device.
- The Manufacturer shall not be liable for any loss or damage arising from use of the product in a manner not in accordance with these instructions.

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. Hovig B, Aandahl EH. A selective method for the isolation of Haemophilus in material from the respiratory tract. Acta Pathol Microb Scand 1969; 77:676-84
2. Gonzales MD, Ledebner NA. Haemophilus. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019.
3. UK Health Security Agency. UK Standards for Microbiology Investigations: Painful and/or discharging ear. London: UKHSA; 2025.
4. Public Health England- UK Standards for microbiology investigations (UK SMI) Investigation of bronchoalveolar lavage, sputum and associated specimens B 57, Issue n° 3.5, 2019
5. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019.

TABLE OF APPLICABLE SYMBOLS

REF Catalogue number	LOT Batch code	IVD In vitro diagnostic medical device	Manufacturer	This way up	For single use only	CE 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	UDI 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	Principle of the method and explanation of the procedure, specimens, limitations of the method, performance characteristics, precautions and warnings, and table of applicable symbols.	2026/03

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

