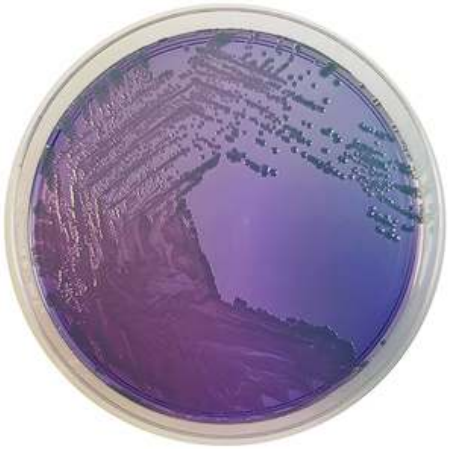
INSTRUCTIONS FOR USE

HERELLEA AGAR

Dehydrated culture medium



Herellea Agar: *A.calcoaceticus*

1 - INTENDED USE

In vitro diagnostic. For isolation, cultivation and differentiation of Gram-negative fermentative and non-fermentative bacteria. It is especially recommended for the differentiation of *Acinetobacter* (formerly *Herellea*) species in urethral and vaginal specimens.

2- COMPOSITION

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

Tryptone	15.00 g
Soy peptone	5.00 g
Sodium chloride	5.00 g
Lactose	10.00 g
Maltose	10.00 g
Bile salts N.3	1.25 g
Bromocresol purple	0.02 g
Agar	16.00 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

Herellea Agar has been formulated by Mandel, Wright and McKinnon[1] in 1964 as a selective medium for enhancing the isolation of *Mima* and *Herellea* organisms in gonorrhoeal specimens in the presence of large numbers of Gram-positive cocci and Gram-negative rods (usually members of the family *Enterobacteriaceae*) frequently encountered in urethral and vaginal discharges.
Herellea Agar is used for isolation, cultivation and differentiation of Gram-negative fermentative and non-fermentative bacteria and it is especially recommended for the differentiation of *Mima polymorpha* and *Herellea vaginicola* (included together in the species *Acinetobacter)* from *Neisseria gonorrhoeae* in urethral and vaginal specimens.[2]
Casein and soy peptones provide nitrogen, carbon and other essential nutrients for bacterial growth. Inhibition of Gram-positive bacteria and *N.gonorrhoeae* is achieved by the incorporation of bile salts n°3. Sodium chloride maintains the osmotic balance of the medium. Lactose and maltose are fermentable carbohydrates: fermenting bacteria produce acid end-products that make the pH indicator (bromocresol purple) turn yellow. *Acinetobacter* organisms do not ferment the carbohydrates and grows with pale lavender colonies, the same colour of the medium. All acid-producing colonies are yellow, surrounded by a yellow zone.[1]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 62 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes, cool to approximately 47-50ºC and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale violet, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Herellea Agar	Dehydrated medium	4015432	500 g (8,1)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Herellea Agar is used for the bacteriological processing of clinical specimens such as urethral and vaginal specimens.[1,2,] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
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 inoculated plates, in aerobic conditions at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:

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Acinetobacter spp. do not ferment lactose and maltose and grow with colonies of the same colour of the medium, sometimes with a slight colour change to a more intense violet.
Lactose and maltose fermenting *Enterobacteriaceae* grow with yellow colonies surrounded by yellow halos.

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, 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.[8]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
A.calcoaceticus ATCC 19606	35-37°C / 18-24H / A	good growth, pale lavender colonies
E.coli ATCC 25922	35-37°C / 18-24H / A	good growth, yellow colonies and medium
S.aureus ATCC 25923	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Herellea Agar is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with 3 target strains: *A.calcoaceticus* ATCC 19606 and two *A.baumanni*, clinical isolates. The colonies of target strains appear with the same colour of the medium; the amount of growth on the plates is evaluated and shall be comparable in both batches.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with the following strains: *E.coli* ATCC 25922, S.Typhimurium ATCC 14028 and *P.aeruginosa* ATCC 14207; the colonies of *E.coli* and *S*.Typhimurium are yellow with yellow halos, the colonies of *P.aeruginosa* are grey-green with diffusible pigment; the amount of growth on the plates is evaluated and shall be comparable in both batches.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of non-target strains**:** *L.acidophilus* clinical isolate, *B.subtilis* ATCC 6633*, E.faecalis* ATCC 19433, *S.aureus* ATCC 25923 . The growth of non-target strains is completely inhibited.

13 - LIMITATIONS OF THE METHOD

- *Pseudomonas* and *Proteus* spp. are not inhibited; however, they do not produce acids. *Proteus* colonies are colourless, *Pseudomonas* colonies are grey-green with a diffusible pigment.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- 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.

14 - 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- 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 media 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.
- 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.

15 - 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).

16 - REFERENCES

1. Mandel AD, Wright K, McKinnon JM. Selective medium for isolation of Mima and Herellea organisms. J Bacteriol 1964; 88:1524
2. Ronald M. Atlas, James W. Snyder. Handbook of Media for Clinical and Public Health Microbiology. CRC Press, 2014
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/07
Revision 2	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/03
Revision 3	Removal of obsolete classification	2023/04

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

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