INSTRUCTIONS FOR USE

HERELLEA AGAR

Ready-to-use plates

Herellea Agar: *A.calcoaceticus*

1 - INTENDED USE

In vitro diagnostic device. 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 *

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
Purified water	1000 mL

*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 grow with pale lavender colonies, the same colour of the medium. All acid-producing colonies are yellow, surrounded by a yellow zone.[1]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	violet, limpid
Final pH at 20-25°C	6.8 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Herellea Agar	Ready-to-use plates	549994	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, ancillary culture media and reagents for the identification of the colonies.

7 - 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.

8- 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.

9 - READING AND INTERPRETATION

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

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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.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
A.calcoaceticus ATCC 19606	34-37°C / 18-24H / A	good growth, pale lavender colonies
E.coli ATCC 25922	34-37°C / 18-24H / A	good growth, yellow colonies and medium
S.aureus ATCC 25923	34-37°C / 18-24H / A	inhibited

A: aerobic incubation; 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 Herellea Agar and of the raw material used for the production of prepared plates (dehydrated Herellea Agar REF 401543) are 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.
Specificity 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, *P.mirabilis* ATCC 12453, 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.mirabilis* are colourless, 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 29212, *S.aureus* ATCC 25923 . The growth of non-target strains is completely inhibited.

12 - 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.

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. 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 of the product are available on the website www.biolifeitaliana.it.
- 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. 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	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	2020/10
Instructions for Use (IFU) - Revision 2	Removal of obsolete classification	2023/04

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

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