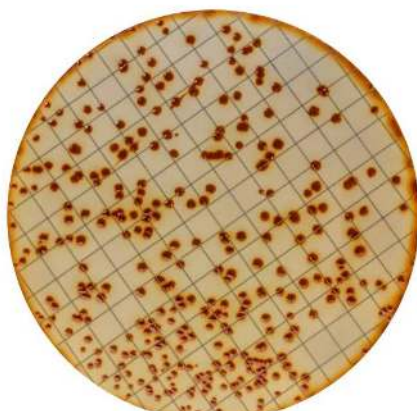




CLARK AGAR

Dehydrated culture medium and ready to use medium in plates

Clark Agar: *Pseudomonas aeruginosa*

1 - INTENDED USE

For the detection and enumeration of heterotrophic iron-precipitating bacteria.

2 - COMPOSITION *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Ammonium sulfate	0.5 g
Sodium nitrate	0.5 g
Magnesium sulphate 7H ₂ O	0.5 g
Dibasic potassium phosphate	0.5 g
Ferrous ammonium citrate(III)	10.0 g
Agar	15.0 g

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Biocorrosion is a phenomenon that is usually attributed to certain bacterial species, such as ferrobacteria and sulphate-reducing bacteria. However, any bacterium capable of producing acidic extracellular polymers can adsorb positively charged iron hydroxides when placed in neutral or slightly acidic conditions¹. Clark Agar, formulated according to the UNICHIM MU1038/2:2002 method, is indicated for the isolation of this type of bacteria in mains water and in sediment samples from pipes. The synthetic composition of Clark Agar provides all the substances necessary for bacterial growth, while the high concentration of ferrous ammonium citrate (III) is the indicator for the iron precipitation reaction.

4- DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 27 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 47-50°C and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	brown, fine, homogeneous, free-flowing powder
Prepared plates appearance	brick color, clear
Final pH (at 20-25°C)	7.2 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Clark Agar	Dehydrated medium	4013152	500 g (18,5 L)
		4013154	5 kg (185 L)
Clark Agar	Ready-to-use plates	491315	3 x 10 plates ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, filter membranes, ancillary culture media and reagents.

8 - SPECIMENS

Mains water, sediment taken from pipes. For the collection, storage, transport and preparation of samples, follow the rules of good laboratory practice. Consult reference standards for sampling methods and sample handling procedures².

9 - TEST PROCEDURE

Filter 100mL of sample through a sterile membrane (0.45 µm pore size). Place the membrane filter on the surface of the medium and make sure there is no air trapped underneath. In the case of sediment sample, weigh it, note the exact weight and suspend in an appropriate volume of sterile water. Leave at room temperature for 10 minutes and spread 0.1 mL of the suspension on Clark Agar or inoculate by looping.

Incubate the plates at 22 ± 1°C for 7-10 days.

10 - READING AND INTERPRETATION

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

Consider colonies that have a brick red color to be ferroprecipitating bacteria. Isolated colonies can be subjected to biochemical identification tests. In the case of a liquid sample, express the result in CFU/100mL. For samples made up of sediments, express the result as CFU/g, also considering the quantity of water used to suspend the sample, or note the qualitative result³.

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.





CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>P. aeruginosa</i> ATCC 27853	22°C / 7 days / A	Good growth, rust color colonies
<i>S. marcescens</i> ATCC 8100	22°C / 7 days / A	Good growth, rust color colonies
<i>E. aerogenes</i> ATCC 13048	22°C / 7 days / A	Good growth, rust color colonies
<i>B. subtilis</i> ATCC 6633	22°C / 7 days / A	Good growth, rust color colonies

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

12- PERFORMANCES CHARACTERISTICS

Before release for sale, representative samples of all batches of dehydrated and ready-to-use Clark Agar on plates (Test Batch – TB) are tested for performance characteristics, comparing the results with a previously approved batch (Reference Batch – RB).

Productivity is tested using a quantitative assay with the target strains *P. aeruginosa* ATCC 27853, *S. marcescens* ATCC 8100, *E. aerogenes* ATCC 13048 and *B. subtilis* ATCC 6633; the filter membranes on the medium are inoculated with decimal dilutions in physiological solution of a suspension of colonies and the plates are incubated at 22°C for 7 days. Colonies are enumerated on the Test Batch (TB) and Reference Batch (RB) and the productivity ratio is calculated ($Pr = \frac{CFU_{TB}}{CFU_{RB}}$). If Pr is ≥ 0.7 and if the morphology and color of the colonies are typical, the results are considered acceptable and within specifications.

13 - PRECAUTIONS AND WARNINGS

- These products are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated medium is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- Apply good manufacturing practices in the plates preparation process.
- 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplements and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet 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

Dehydrated medium

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).

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+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°C /+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).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/flasks) and the applied storage conditions (temperature and packaging).

15 - REFERENCES

1. Fontani N., Pedroni M. *Biologia Ambientale* n°6/1994-Batteri implicati nei fenomeni di corrosione; 1994
2. ISO 5667-5:2006 Water quality – Sampling – Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems.
3. UNICHIM MU1038/2:2002, Acque destinate al consumo umano-ricerca dei batteri coinvolti nei fenomeni corrosive (analisi culturale). 2002: 11

TABLE OF APPLICABLE SYMBOLS

or REF Catalogue number	Batch code	Manufacturer	For single use only	This side up	Store in a dry place
Temperature imitation	Content sufficient for <n> tests	Consult Instruction s for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First version	2024/06

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

