m-AEROMONAS SELECTIVE AGAR BASE (HAVELAAR)
AEROMONAS SELECTIVE SUPPLEMENT
Dehydrated culture medium and selective supplement

Aeromonas Selective Agar (Havelaar): *A.hydrophyla* colonies on a membrane filter

1 - INTENDED USE
Selective and differential medium for the detection of *Aeromonas* in finished water by membrane filtration.

2 - COMPOSITION *
AEROMONAS SELECTIVE AGAR BASE (HAVELAAR)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	5.00 g
Yeast extract	2.00 g
Dextrin	11.40 g
Sodium chloride	3.00 g
Potassium chloride	2.00 g
Magnesium sulphate	0.10 g
Ferric chloride	0.06 g
Sodium desoxycholate	0.10 g
Bromothymol blue	0.08 g
Agar	13.00 g

AEROMONAS SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Ampicillin	5 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Aeromonas is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family *Enterobacteriaceae*. The significance of *Aeromonas* species as human pathogens is getting increasing attention.[1] The organisms are ubiquitous in fresh and brackish water[2], are considered as potential food-poisoning agents [3] and are responsible for, and are implicated in, a number of intestinal and extra-intestinal infections in humans as well as other animals.[4]
m-Aeromonas Selective Agar Base supplemented with ampicillin, corresponds to the medium described by Havelaar et al.[5,6] Supplemented with ampicillin and vancomycin, it is recommended by United States Environmental Protection Agency (USEPA Method 1605), for the detection of *Aeromonas* in finished water by membrane filtration.[7]
Recovery from pure cultures and environmental samples is optimal and specificity is high.[5] The use of ampicillin suppresses adequately the background flora without having any decrease in the *Aeromonas* recovery. Strains sensitive to 10 mg/l of ampicillin appear to occur at a frequency of 1% or less.[5] Vancomycin increases selectivity properties due to its inhibitory action on Gram-positive bacteria.

4 - DIRECTIONS FOR MEDIUM PREPARATION
Suspend 18.35 g in 500 mL of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and, under aseptic conditions, add the contents of one vial of Aeromonas Selective Supplement-Ampicillin (REF 4240012) reconstituted with 5ml of sterile distilled water. Mix well and distribute into sterile 55 mm dishes.
USEPA methods require the addition of vancomycin hydrochloride: reconstitute one vial of Vancomycin Selective Supplement CSB (REF 4240057C) with 5 mL of sterile purified water; to 500 mL of medium prepared as described above with ampicillin, add 1 mL of Vancomycin Selective Supplement CBS (final concentration of vancomycin: 2 mg/L).

5 - PHYSICAL CHARACTERISTICS

Aeromonas Selective Agar Base (Havelaar)	
Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Prepared plates appearance	green-light blue, limpid, limpid
Final pH at 20-25 °C	8.0 ± 0.2
Aeromonas Selective Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
m-Aeromonas Selective Agar Base	Dehydrated medium	4010192	500 g (13.6 L)
Aeromonas Selective Supplement-Ampicillin	Freeze-dried selective supplement	4240012	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, 55 mm dishes, membrane filters, Vancomycin Selective Supplement CSB (REF 4240057C), ancillary culture media and reagents.

8 – SPECIMENS
Samples representative of the drinking water distribution system. Consult the appropriate reference for sample collection, preservation, and storage.[7]

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9 - TEST PROCEDURE

Appropriate volumes or decimal dilutions of the samples are filtered using membrane filters (0.45 µm pore size), and the filters are transferred onto the medium surface. Incubate in aerobic conditions at 30°C[5] or 35°C[7] for 24 ± 2 hours

10 - READING AND INTERPRETATION

After incubation, *Aeromonas* colonies show a visible yellow colour (dextrin fermentation). The detection of dextrin fermentation is considered to be highly specific and until now no dextrin negative *Aeromonas* strains have been found.[5]
Confirm the presumptive detection with standard biochemical tests: trehalose fermentation, oxidase test and production of indole. Any presumptive colony that is positive for oxidase, ferments trehalose, and produces indole is considered to be *Aeromonas*.[7]

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
A.hydrophila ATCC 7966	35°C / 24 H/ A	growth with yellow colonies
E.coli ATCC 11775	35°C / 24 H/ A	no growth

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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated m-Aeromonas Selective Agar Base supplemented with ampicillin (Test Batch: TB) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative technique with the target strains *A.hydrophila* ATCC 7966 and *A.hydrophila* ATCC 35654.
The membrane filters on the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35°C for 24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: UFC_{TB}/UFC_{RB}) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (yellow colonies) the results are considered acceptable and conform to the specifications
The 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 strains *E.coli* ATCC 25922, *E.aerogenes* ATCC 13048, *S.marcescens* ATCC 8100, *P.aeruginosa* ATCC 14207, *E.faecalis* ATCC 19433, *S.aureus* ATCC 25923. *E.coli*, *S.aureus* and *E.faecalis* are totally inhibited while the growth of the other non-target strains is partially inhibited

13-LIMITATIONS OF THE METHODS

- A very low percentage of *Aeromonas,* susceptible to 10 mg/L of ampicillin, may be inhibited on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, suitable identification tests should be performed.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement 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.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- 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 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.
- Be careful when opening the metal ring of the vials to avoid injury.
- The supplement is sterilized by membrane filtration
- 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 supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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.

15 - 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).

Freeze-dried supplement
Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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/bottles) and the applied storage conditions (temperature and packaging). According to USEPA method[7], the prepared plates with ampicillin and vancomycin should be stored in a tight-fitting container (i.e., sealed plastic bag) at a temperature of 1 to 5°C for no longer than 14 days.

16 - REFERENCES

1. Holmberg SC, Farmer JJ. Aeromonas hydrophila and Plesiomonas shigelloides as causes of intestinal infections Rev Inf Dis. 1984; 6: 633-639
2. Graf J (editor). (2015). Aeromonas. Caister Academic Press.
3. Palumbo SA, Maxino F, Williams AC Buchanan RL, Thayer DW.. Starch-Ampicillin Agar for the Quantitative Detection of Aeromonas hydrophila. App Environ Microbiol. 1985; 50:1027-1030.
4. Parker JL, Shaw JG. Aeromonas spp. clinical microbiology and disease. J Infect . 2011 Feb; 62(2):109-18.
5. Havelaar AH, During M, Versteegh JFM. Ampicillin-dextrin agar medium for the enumeration of Aeromonas species in water by membrane filtration. J App Bact 1988; 62: 279-287.
6. Havelaar AH, Vonk M. The preparation of ampicillin dextrin agar for the enumeration of Aeromonas in water. Letters App Bact 1988; 7: 169-171
7. United States Environmental Protection Agency (USEPA), Method 1605: Aeromonas in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V). October 2001.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
emperature mitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/04

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

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