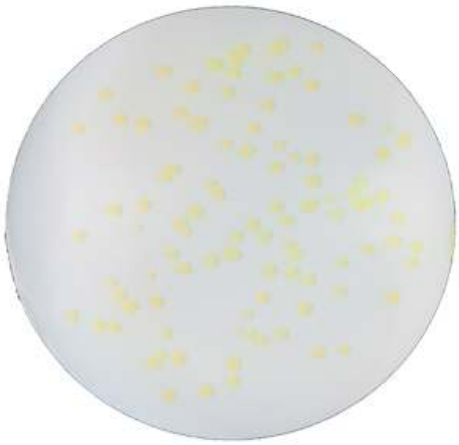
PSEUDOMONAS AGAR BASE
CN PSEUDOMONAS SUPPLEMENT
CFC PSEUDOMONAS SUPPLEMENT
PP PSEUDOMONAS SUPPLEMENT
PSEUDOMONAS CN SELECTIVE AGAR

Dehydrated culture medium, selective supplements, ready-to use plates



Pseudomonas CN Selective Agar: colonies of *P. aeruginosa* on a membrane filter

PSEUDOMONAS CN SELECTIVE AGAR (READY TO USE PLATES 55 AND 90 MM)

Pseudomonas Agar Base	1000 mL
Cetrimide	200 mg
Nalidixic Acid	15 mg
Glycerol	10 mL

1 - INTENDED USE

For the isolation and enumeration of *Pseudomonas* spp. in waters, foodstuffs and environmental samples.

2 – COMPOSITIONS*

PSEUDOMONAS AGAR BASE – DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of gelatin	16.0 g
Acid digest of casein	10.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Agar	11.5 g

CN PSEUDOMONAS SUPPLEMENT (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cetrimide	100 mg
Nalidixic acid	7.5 mg

CFC PSEUDOMONAS SUPPLEMENT (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cetrimide	5 mg
Fusidic acid	5 mg
Cephalosporin	25 mg

PP PSEUDOMONAS SUPPLEMENT (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Penicilin G	50,000 UI
Pimaricin	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

Pseudomonas aeruginosa is an opportunistic environmental pathogen characterized by a high degree of adaptability, capable of growing in waters with very low nutrients concentrations and surviving in disinfected water.[1] *Pseudomonas* spp. are among the most frequently reported psychotropic bacteria in raw milk and are the most common cause of the meat spoilage at refrigeration temperature.[2]
Pseudomoas Agar Base is a modification of King's A Medium[3] in which the concentration of peptones is significantly increased and magnesium chloride and potassium sulphate are present to enhance pigments production.
The use of cetrimide at 10 mg/L and nalidixic acid at 15 mg/L, (CN Pseudomonas Supplement), was described by Goto and Enomoto[4] after the studies of Lowbury and Collins[5] on the selective properties of cetrimide against a broad range of Gram-positive and some Gram-negative organisms other than *P.aeruginosa*. The combination of cetrimide and nalidixic acid strongly suppresses the growth of *Klebsiella, Proteus* and *Providencia* spp.
CFC Supplement is based on the formulation devised by Mead and Adams[6] who demonstrated that the reduction of cetrimide to 10 mg/L improved the recovery of pigmented and non- pigmented psychrophilic *Pseudomonas* associated with poultry meat spoilage. To enhance the medium selectivity, they added a cephalosporin and fusidic acid.
PP Supplement is based on the formulation included in ISO 11059[7] and contains penicillin as inhibitory agent for Gram positive bacteria and the antifungal compound pimaricin (natamycin).
Pseudomonas Agar Base supplemented with glycerol and CN Supplement corresponds to the medium recommended by ISO 16266[8] for the isolation and enumeration *of P. aeruginosa* in water samples.
The medium base supplemented with CFC Supplement corresponds to the medium recommended by ISO 13720[7] for the isolation and enumeration of *Pseudomonas* spp. in meat products.
The medium base supplemented with PP Supplement corresponds to the medium recommended by ISO/TS 11059[9] for the isolation and enumeration of *Pseudomonas* spp. in milk and milk products.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 24.5 g in 500 mL of cold purified water and add 5 mL of g Glycerol (REF 421025) for the preparation of CN Pseudomonas Agar (ISO 16266); omit the glycerol for the preparation of CFC and PP Media. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 45-50°C and add the suitable selective supplement as follows:
CN Supplement (ISO 16266) Add the contents of one vial of CN Pseudomonas Supplement (REF 4240046) reconstituted with 2 mL of sterile distilled water/ethanol (1:1), to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.
CFC Supplement (ISO 13720) Add the contents of one vial of CFC Pseudomonas Supplement (REF 4240075) reconstituted with 2 mL of sterile distilled water/ethanol (1:1), to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.
PP Supplement (ISO/TS 11059) Add the contents of one vial of PP Pseudomonas Supplement (REF 4240048) reconstituted with 2 mL of sterile distilled water, to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.

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5 - PHYSICAL CHARACTERISTICS

Pseudomonas Agar Base

Dehydrated medium appearance	white, fine, free-flowing powder
Solution appearance	pale yellow, limpid or slightly opalescent
CN medium: final pH at 20-25 °C (ISO 16266)	7.1 ± 0.2
CFC and PP media: final pH at 20-25 °C (ISO 13720-ISO/TS 11059)	7.2 ± 0.2
CN Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	colourless, limpid
CFC Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	colourless to pale yellow, limpid
PP Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	whitish, turbid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pseudomoas Agar Base	Dehydrated medium	4019602	500 g (10.2 L)
CN Pseudomonas Supplement	Freeze-dried supplement	4240046	10 vials, each for 500 mL of medium
CFC Pseudomonas Supplement	Freeze-dried supplement	4240075	10 vials, each for 500 mL of medium
PP Pseudomonas Supplement	Freeze-dried supplement	4240048	10 vials, each for 500 mL of medium
Pseudomonas CN Selective Agar	Ready to use plates	541960	2 x 10 plates ø 90 mm
Pseudomonas CN Selective Agar	Ready to use plates	491960	3 x 10 plates ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and pipettes, Petri dishes, Erlenmeyer flasks, Glycerol (REF 421025), ancillary culture media and reagents.

8 - SPECIMENS

Water samples, meat and meat products, milk and milk products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[7-9]

9 - TEST PROCEDURE - READING AND INTERPRETATION

Waters (ISO 16266)[8]

1. Filter through a membrane filter of 0.45 µm the appropriate volume of water (bottled water or a spring water: 250 mL, other waters, including pool waters and water for human consumption: 100 mL).
2. Place each membrane on a plate prepared with CN Supplement, ensuring no air is trapped beneath the membrane.
3. Incubate at 36 ± 2 °C for 44 ± 4 h. Examine the membranes for growth after 22 ± 2 h and 44 ± 4 h.
4. Count all colonies that produce green/blue (pyocyanin) colour as confirmed *P.aeruginosa*.
5. Examine the membrane under UV radiation. Count all non-pyocyanin producing colonies and reddish brown pigmented colonies that fluoresce as presumptive *P.aeruginosa* and confirm their identity using the oxidase test (Oxidase Test Strip, REF 191040ST), acetamide broth (Acetamide Broth, REF 5510101), and King's B medium (Pseudomonas Agar F, REF 401961).

Meat products (ISO 13720)[9]

1. Transfer on the surface of one plate prepared with CFC Supplement 0.1 mL of the initial suspension. Repeat this operation with subsequent dilutions taking two other CFC agar plates, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).
2. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dray.
3. Incubate the dishes at 25 °C ± 1 °C for 44 h ± 4 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.
4. Confirm the presence of *Pseudomonas* with oxidase test. Colonies showing a positive oxidase reaction shall be considered as *Pseudomonas* colonies.

Milk and milk products (ISO/TS 11059)[7]

1. Transfer on the surface of the plate prepared with PP Supplement 0.1 mL of the initial suspension. Repeat this operation with subsequent dilutions taking other PP Agar plates, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).
2. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dray.
3. Incubate the dishes at 25 °C ± 1 °C for 48 h ± 2 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.
4. Confirm the presence of *Pseudomonas* with oxidase test and fermentation of glucose on Purple Glucose Agar (REF 401970).
5. Colonies showing a positive oxidase reaction and absence of glucose fermentation shall be considered as *Pseudomonas* colonies.

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
Pseudomonas Agar Base + CN Supplement			
P. aeruginosa	ATCC 10145	36°C / 40-48 H / A	yellow-green, fluorescent colonies
E. coli	ATCC 25922	36°C / 40-48 H / A	inhibited

Pseudomonas Agar Base + CFC Supplement			
P. fluorescens	ATCC 13525	25°C / 40-48 H / A	green-blue, fluorescent colonies
E. coli	ATCC 25922	25°C / 40-48 H / A	inhibited
Pseudomonas Agar Base + PP Supplement			
P. aeruginosa	ATCC 27853	25°C / 40-48 H / A	yellow-green, fluorescent colonies
P. fluorescens	ATCC 13525	25°C / 40-48 H / A	green-blue, fluorescent colonies
E. coli	ATCC 25922	25°C / 40-48 H / A	inhibited

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

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use medium and supplements (Test Batch:TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch and Tryptic Soy Agar.
Incubation time and temperature: Pseudomonas Agar Base+CN Supplement: 36°C for 44-48 hours, Pseudomonas Agar Base+CFC and PP Supplement: 25°C for 40-48 hours. Inoculation technique: spread plate.
Productivity of Pseudomonas Agar Base+CN Supplement is assessed by a quantitative test with the following target strains: *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 10145, *P. aeruginosa* ATCC 9027. The productivity ratio (CFU_{TB}/CFU_{TSA}) shall be ≥ 0.5.
Productivity of Pseudomoas Agar Base+CFC Supplement is assessed by a quantitative test with the target strain *P. fluorescens* ATCC 13525. The productivity ratio (CFU_{TB}/CFU_{TSA}) shall be ≥ 0.5. Moreover, the productivity characteristics are evaluated by semiquantitative ecometric technique with *P. aeruginosa* ATCC 10299, and *P. aeruginosa* ATCC 9027. After incubation the target strains exhibit good growth with typical colonies.
Productivity of Pseudomoas Agar Base+PP Supplement is assessed by a quantitative test with the target strains *P. fluorescens* ATCC 13525 and *P. aeruginosa* ATCC 27853. The productivity ratio (CFU_{TB}/CFU_{TSA}) shall be ≥ 0.5. Moreover, the productivity characteristics are evaluated with semiquantitative ecometric technique with *P. aeruginosa* ATCC 10299 and *P. aeruginosa* ATCC 9027. After incubation the target strains exhibit good growth with typical colonies
Selectivity properties of media are assessed with the following non-target strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. mirabilis* ATCC 12413, *E. faecalis* ATCC 19433. All types of media with the selective supplements exhibit a total inhibition of non-target strains.

12 - LIMITATIONS OF THE METHOD

- Where large numbers of presumptive *P. aeruginosa* are isolated, the spreading nature of colonies can hinder precise quantitative assessment.[8]
- The pigment pyocyanin (blue-green) is produced by more than 90 % of *P. aeruginosa* strains.[8]
- The identification of *Pseudomoas* spp. or *P. aeruginosa* should be confirmed by suitable tests.

13 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready to use plates 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 supplements shall be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. CN, CFC and PP supplements are classified as hazardous. Before use, consult the 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- The selective supplements are sterilised by membrane filtration.
- Be careful when opening the metal rings of the supplements vials to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplements and sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements 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 products for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

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 2-8°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).
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).

Selective supplements

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 and the applied storage conditions (temperature and packaging).

According to ISO 16266 the self-prepared CN agar plates can be kept in the dark protected from desiccation for not more than 4 weeks at 5 °C ± 3 °C. Do not keep the agar molten for more than 4 hours. [8]

According to ISO 13720 the self-prepared CFC agar plates can be kept in the dark protected from desiccation for not more than 4 weeks at 5 °C ± 3 °C.[9]

According to ISO 11059, the self-prepared PP agar plates can be kept in the dark at 5 °C ± 3 °C for no longer than 1 day.[7]

15 - REFERENCES

1. Briancesco R, Paduano S, Semproni M, Vitanza L, Bonadonna L. Behavior of Pseudomonas aeruginosa and Enterobacter aerogenes in Water from Filter Jugs. Int. J. Environ. Res. Public Health 2020; 17: 8263.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
3. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
4. Goto S. Enomoto S. Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of Pseudomonas aeruginosa. Jpn J Microbiol 1970; 14: 65-72
5. Lowbury EJ, Collins AG. The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J Clin Pathol 1955; 8:47-8.
6. Mead GC, Adams BW. A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. Br Poult Sci 1977; 18: 661-70.
7. ISO/TS 11059:2009 Milk and milk products — Method for the enumeration of Pseudomonas spp.
8. ISO 16266:2006 Water quality – Detection and enumeration of Pseudomonas aeruginosa by membrane filtration.
9. ISO 13720:2010 Meat and meat products — Enumeration of presumptive Pseudomonas spp

TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 5	Updated layout and content	2022/10

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

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