Instructions for Use

REF - KM0079 **Pseudomonas Agar Base**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0079 *Pseudomonas* Agar Base with the addition of supplements, is a selective medium for the isolation of *Pseudomonas* species primarily from clinical, food, water, and environmental samples.

2. Composition*

Ingredient	g/L
Acid casein	10
Gelatine peptone	16
Potassium sulphate	10
Magnesium chloride	1.4
Agar	11

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

The medium can be made selective for *Pseudomonas aeruginosa* by the addition of E&O LS0006 Pseudomonas Selective Cetrimide and Nalidixic Acid Supplement [(1)] which complies to ISO 16266:2006 [(2)] and ISO 11133:2014 [(3)]. Alternatively, the medium can be made selective for *Pseudomonas* species by the addition of E&O LS0026 Pseudomonas CFC Selective Supplement [(4)] which complies to ISO 13720:2010 [(5)] and can be used as a primary isolation medium according to Public Health England**'s** UK Standards for Microbiology Investigations [(6)].

4. Principle

The gelatine peptone and acid hydrolysed casein acts as a source of nitrogen, carbon, and other essential nutrients. Identification is achieved using the unique ability of *P. aeruginosa* to synthesise the iron chelating pigments pyoverdin and pyocyanin which combine to produce the characteristic green colonies of *Pseudomonas aeruginosa* [(7)]. Production of these pigments is stimulated by the presence of magnesium and potassium ions in the medium. The presence of blue/green or brown pigmentation or fluorescence is indicative of presumptive *Pseudomonas* species It should be noted however that further testing must be conducted to confirm the full identity of the organism.

5. Preparation Instructions

Suspend 48.4g/L of dried culture media in deionised/purified water, add 10ml/L of glycerol and mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C. Aseptically add the appropriate amount of either Pseudomonas CN Supplement (E&O LS0006) or Pseudomonas CFC Selective Supplement (E&O LS0026).

Mix gently but thoroughly before aseptically dispensing the specified volume into appropriate sterile containers.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Pale straw firm gel
pH	N/A	7.1 ± 0.2

7. Materials Provided

KM0079 is available in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	**Product Format**
KM0079-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0079-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0079-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0079 *Pseudomonas* Agar Base is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples
- Clinical: a variety of samples, including human skin, throat, and stool samples
- Water Samples: Potable waters, water from heating systems, cooling towers and air conditioning systems

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Follow local, national, or international standards or guidelines relevant to sample type.

Prepare KM0079 according to Section 5, adding the selective supplement required.

Based on sample type and information provided, determine whether the clinical specimen requires pre-enrichment prior to inoculation to the prepared medium.

Inoculate plated medium directly with the sample, or subculture onto plated medium after incubation in enrichment broth where required.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:10 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, medium should be inoculated with the specimen and incubated at 30 ± 1°C aerobically for 24-48 hours.

Water Specimens:

Filtration: A suitable volume of the sample is passed through a filter membrane of 0.45µm pore and the membrane is then placed on the surface of the medium, ensuring there are no air bubbles underneath, before incubating at 30 ± 1°C aerobically for 24-48 hours.

Spread plate: 0.5ml of the specimen, or pre-enriched sample, is inoculated directly onto the medium and streaked across the agar surface using a sterile loop or automated plate streaker, before incubating at 30 ± 1°C aerobically for 24-48 hours.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 35-37°C aerobically for 24-72 hours [6].

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
P. aeruginosa (NCTC 12903)	30 ± 1°C aerobically for 24-48 hours	Growth: Blue/green pigmented colonies
B. cepacia (NCTC 10661)	30 ± 1°C aerobically for 24-48 hours	Growth: Grey colonies
P. aeruginosa (NCTC 12924)	30 ± 1°C aerobically for 24-48 hours	Growth: Blue/green pigmented colonies
E. coli (NCTC 12241)	30 ± 1°C aerobically for 24-48 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify the performance of KM0079 *Pseudomonas* Agar Base, dehydrated culture media samples were used to prepare Pseudomonas CFC Selective Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Prepared plates were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 30 ± 1°C aerobically for 24-48 hours. All samples of prepared media produced expected recovery and morphology of the required test organisms: *Pseudomonas aeruginosa* (NCTC 12903), *Burkholderia cepacia* (NCTC 10661) and *Pseudomonas aeruginosa* (NCTC 12924) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0079 *Pseudomonas* Agar Base, meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Media

- Some strains of *P. aeruginosa* may fail to produce pyocyanin.
- Although unusual, *Enterobacteriaceae* may also grow.
- Biochemical, serological, molecular, or spectrometric testing using pure cultures may be necessary for complete identification.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0079 Material Safety Data Sheet. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Goto, S., and Enomoto, S. (1970): Nalidixic Acid Cetrimide Agar. A New Selective Plating Medium for the Selective Isolation of Pseudomonas aeruginosa. Japan. J. Microbiol. 14: pp.65-72.
2. International Organization for Standardization (2006) 16266:2006 Water quality - Detection and enumeration of Pseudomonas aeruginosa - Method by membrane filtration. Geneva: ISO.
3. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.
4. Mead, G.C. and Adams, B.W. (1977): A selective medium for the rapid isolation of Pseudomonas associated with poultry meat spoilage. Br. Poult. Sci. 18: pp.661-670.
5. International Organization for Standardization (2010) 13720:2010 Meat and meat products — Enumeration of presumptive Pseudomonas spp. Geneva: ISO.
6. Public Health England (2015) Identification of *Pseudomonas* species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 17(3). London: Standards Unit, Microbiology Services.
7. King, E., Ward, M. and Raney, D. (1954) Two simple media for the demonstration of pyocyanin and fluorescin. The Journal of laboratory and clinical medicine, 44(2), pp.301-307.

Version History*

001 07/08/2023 - New Document Created
002 04/10/2023 - Updates made to section 14
003 25/03/2025 - Update based on new formatting and device file

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

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TABLE OF APPLICABLE SYMBOLS

REF o 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