

NATIONAL STANDARD METHOD

ENUMERATION OF *PSEUDOMONAS AERUGINOSA* BY MEMBRANE FILTRATION

W 6

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

ENUMERATION OF *PSEUDOMONAS AERUGINOSA* BY MEMBRANE FILTRATION

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AMENDMENT PROCEDURE

Controlled document reference	W 6
Controlled document title	Enumeration of <i>Pseudomonas aeruginosa</i> by membrane filtration

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
6/ 21.12.07	2.3	3	6	Definitions	Amended to include the chemical name for cetrimide and ability to hydrolyse casein when sub-cultured to milk cetrimide agar
				Equipment	Updated to include UV lamps 360 ± 20nm and graduated filter funnels up to 250mL
			7	Culture media and reagents	Quarter strength ringer's solution removed.
			7	Sample processing	Updated Table of confirmatory tests amended.
			10	Reference Facilities; Acknowledgments and Contacts	New sections added
			11	Flowchart	Updated
			12	References	Updated

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SCOPE OF DOCUMENT

The method gives general guidelines for the enumeration or detection of *Pseudomonas aeruginosa* in all types of water samples by the membrane filtration technique. It is generally applicable to water with a low background flora eg water intended for human consumption and pool waters.

INTRODUCTION

Background

The presence of *P. aeruginosa* in potable water is undesirable as subsequent growth is often associated with deterioration in quality including colour, turbidity, taste and odour. However, the enumeration of *P. aeruginosa* is not recommended as a routine procedure although it may be of value in the investigation of consumer complaints² and is required when testing mineral waters for compliance with the Natural Mineral Waters Regulations³.

When water of exceptional bacterial purity is required such as used for pharmaceutical products, it is useful to examine samples for *P. aeruginosa*. It is also useful to examine whirlpools, spa pools, hydrotherapy pools and swimming pools for two reasons: folliculitis and ear infections caused by *P. aeruginosa* have been associated with each of these recreational sources; and the presence of *P. aeruginosa* is often an early indication of a breakdown in the disinfection process due to its increased resistance compared with other indicator organisms. It cannot be used as an indicator of faecal pollution. In certain circumstances it may be the cause of some opportunistic infections in man, especially debilitated patients. Its presence in drinking water, bottled water, swimming pools and hospital water supplies is therefore considered undesirable.

Standards and guidelines for *P. aeruginosa* are given for natural mineral waters sampled within 12 hours of bottling³, hydrotherapy pools⁴ and spa pools⁵. This method is based on the method described in the Microbiology of Drinking Water 2002 document² and BS EN 12780:2002⁶.

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1 DEFINITIONS

For the purpose of this method the following definition applies:

Pseudomonas aeruginosa

Micro-organisms that grow on selective media containing cetrimide (cetyl trimethylammonium bromide) and produce pyocyanin, or micro-organisms that grow on selective media containing cetrimide, are oxidase positive, fluoresce under UV light 360 ± 20 nm and hydrolyse casein when sub-cultured to milk cetrimide agar.

2 PRINCIPLE

A measured volume of the sample or a dilution of the sample is filtered through a membrane that is capable of retaining *P. aeruginosa*. The membrane is incubated on a selective/differential agar and characteristic colonies are counted. Confirmatory tests are carried out where necessary and the result is calculated as the colony count per 250 mL for mineral and bottled waters and per 100 mL for other waters.

3 SAFETY CONSIDERATIONS⁷⁻¹⁷

Normal microbiology laboratory precautions apply.

3.1 SAMPLE TRANSPORT AND STORAGE

Compliance with current postal and transportation regulations is essential.

3.2 SAMPLE PROCESSING

- When the UV lamp is in use it is strongly advised that gloves and either goggles or a face shield suitable for use with 360 ± 20 nm UV emitting sources are worn

The above guidance should be supplemented with local COSHH and risk assessments

4 EQUIPMENT

Usual laboratory equipment and in addition:

- Membrane filtration manifold
- Filter funnels graduated appropriately up to 250mL
- Pyrex vacuum flask with protective jacket: large volume eg 5 L or equivalent
- Vacuum pump with moisture trap or protective filter, or alternative vacuum source
- Stainless steel flat tipped forceps
- Boiling waterbath (instrument steriliser)
- Incubators: $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- Ultra violet lamp (long wave UV 360 ± 20 nm)
- Petri dishes
- Cellulose ester 0.45 μm pore size gridded filters
- Automatic pipettors and associated sterile pipette tips capable of delivering up to 10 mL and 1 mL volumes
- Pipettes (sterile total delivery) 10 mL and 1 mL graduated in 0.1 mL volumes (optional)

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5 CULTURE MEDIA AND REAGENTS

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

Peptone saline diluent (maximum recovery diluent)

Peptone	1.0 g
Sodium chloride	8.5 g
Water	1 L
pH 7.0 ± 0.2 at 25°C	

Pseudomonas agar + CN supplement

Acid hydrolysed peptone or casein hydrolysate	10.0 g
Gelatine peptone	16.0 g
Potassium sulphate (anhydrous)	10.0 g
Magnesium chloride (anhydrous)	1.4 g
Glycerol	10.0 mL
Cetrimide	200 mg
Nalidixic acid, sodium salt	15 mg
Agar	11.0 g
Water	1 L
pH 7.1 ± 0.2 at 25°C	

Milk agar with cetrimide

Yeast extract	0.075 g
Peptone	2.5 g
Sodium chloride	1.25 g
Skimmed milk powder	100.0 g
Cetrimide	0.3 g
Agar	15 g
Water	1 L
pH 7.2 ± 0.2 at 25°C	

Oxidase reagent (Prepare fresh as required or use a commercial equivalent)

Tetramethyl- <i>p</i> -phenylenediamine hydrochloride	0.1 g
Water	10 mL

6 SAMPLE PROCESSING

6.1 SAMPLE PREPARATION AND DILUTIONS

Water samples should be received and handled as described in [WSOP 1 - General technique for the detection and enumeration of bacteria by negative pressure membrane filtration](#). Samples should be examined as soon as is practicable on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

Following the procedures laid down in [WSOP 1 - General technique for the detection and enumeration of bacteria by negative pressure membrane filtration](#) select suitable volumes for analysis and prepare any necessary dilutions.

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6.2 FILTRATION AND INCUBATION

Following the procedures laid down in [NSM W 1 Section 5](#) filter a measured volume of sample through the membrane.

For natural mineral and bottled waters filter 250 mL water, for other potable quality waters and pool waters filter volumes of 100 mL.

Place the membrane onto *Pseudomonas* agar containing cetrime and nalidixic acid and place in an incubator at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

6.3 COUNTING OF COLONIES

Examine plates after 22 hours \pm 2 hours and 44 hours \pm 4 hours incubation.

Count all colonies that produce a green or blue (demonstrating pyocyanin production), or reddish brown pigment and those which fluoresce with UV light. Exposure of colonies to daylight for 2-4 hours enhances pigment production.

Note: When there is a moderately heavy growth of *P. aeruginosa* and other organisms on the membrane, colonies adjacent to pyocyanin producing colonies of *P. aeruginosa* can also appear green after 44 hours \pm 4 hours incubation making the interpretation of the count difficult. Observing the plates after 22 hours \pm 2 hours assists in the interpretation in these instances. If in doubt, all green colonies can be confirmed.

6.4 CONFIRMATORY TESTS

Colonies that clearly produce pyocyanin (green/blue pigmented) on the membrane are considered to be *P. aeruginosa* and require no further testing. Other colonies which fluoresce or are red/brown require confirmation. If more than one volume or dilution has been filtered, proceed if possible with the membrane yielding 20 - 80 colonies.

To confirm other colonies, select as described in [NSM W1 Section 5](#). Sub-culture from the membrane onto a milk cetrime agar (MCA) plate and incubate at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 22 hours \pm 2 hours. Examine the plates for growth, pigment, fluorescence and casein hydrolysis (clearing of the milk medium around the colonies). If pigment production is poor expose the MCA to daylight at room temperature for 2 - 4 hours so as to enhance pigment production and re-examine.

Perform an oxidase test using colonies from the MCA plate. Moisten a piece of filter paper in a Petri dish with 2 – 3 drops of freshly prepared oxidase reagent or equivalent. Using a stick, glass rod or platinum/plastic (not nichrome) loop transfer a colony of the test organism to the filter paper and rub it on the moistened area. A positive reaction is indicated by the appearance of a dark purple colour within 10 seconds. No colour change or a purplish colour which develops later are both negative reactions.

P. aeruginosa is oxidase positive, hydrolyses casein and produces pyocyanin and/or fluorescence. Occasionally atypical non-pigmented variants of *P. aeruginosa* occur. A pyocyanin negative, casein hydrolysis positive, fluorescence positive culture shall be regarded as *P. aeruginosa*.

Interpretation of results is as follows:

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Colony on CN agar	Oxidase test	Fluorescing on MCA	Caseinolytic on MCA	Confirmed <i>P. aeruginosa</i>
Blue or Green	NT	NT	NT	Yes
Fluorescing and not pigmented	+	+	+	Yes
Reddish brown non fluorescing	+	+	+	Yes

NT – Not tested

7 CALCULATION OF RESULTS

Calculate the presumptive count of test organism as follows:

$$\text{Presumptive count/100 mL} = \frac{\text{Number of colonies counted}}{\text{Volume tested}} \times 100$$

Following the procedure described in [NSM W 1 Section 7](#), calculate the number of confirmed *P. aeruginosa* present in the original sample.

For natural mineral and bottled waters calculate the count per 250 mL.

8 REPORTING

Report the results using the procedure described in [NSM W 1 Section 9](#).

If *Pseudomonas aeruginosa* is not detected, report as:

‘Not detected in 250 mL’ or ‘Not detected in 100 mL’ as appropriate

If the test organism is present, report as:

‘a in 250 mL’ or ‘a in 100 mL’ as appropriate

Where **a** is the confirmed count.

For natural mineral waters the result is reported as the count in 250 mL.

9 QUALITY CONTROL

Membrane filtration

When the membrane filtration technique is used internal quality control procedures must be carried out at least once daily depending on the workload of the laboratory. If more than one batch of medium is used in a session it is necessary to repeat the quality control test for each batch.

The quantitative internal quality controls should be carried out using suspensions of positive and negative control organisms known to contain less than 100 colony forming units in the volume filtered.

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Positive control
P. aeruginosa NCTC 10662

Negative control
Escherichia coli NCTC 9001

Blank control
Filter 100 mL of sterile distilled water or peptone saline diluent using the same funnel as used for the positive control following disinfection.

Incubate all tests in parallel with routine tests.

Confirmatory test
Inoculate each batch of medium used on the day of testing with the control organisms and incubate in parallel with the routine tests.

Milk cetrimide agar

P. aeruginosa NCTC 10662 Growth +, casein hydrolysis +, pyocyanin + and fluorescence +

E. coli NCTC 9001 Growth +, casein hydrolysis -, pyocyanin -, fluorescence -

Incubate all tests in parallel with routine tests.

10 REFERENCE FACILITIES

N/A

11 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Water Working Group for Standard Methods (http://www.hpa-standardmethods.org.uk/wg_water.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

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FLOWCHART SHOWING THE ENUMERATION OF *PSEUDOMONAS AERUGINOSA* BY MEMBRANE FILTRATION

Transport to laboratory at 2°C – 8°C out of direct sunlight in suitable containers



Store at 2°C – 8°C in the dark and examine on the day of collection if possible otherwise within 24 hours of collection



Mix sample well and make any necessary dilutions



Filter



Place membrane on *Pseudomonas* agar containing cetrimide and nalidixic acid



Incubate at 37°C ± 1°C for 22 hours ± 2 hours. Count green or blue colonies and re-incubate for a further 22 hours ± 2 hours



Count colonies that produce a green, blue or reddish brown pigment and those which fluoresce under the ultra violet lamp



Sub-culture non-pyocyanin producing (blue/green) colonies to MCA. Incubate at 37°C ± 1°C for 22 ± 2 hours. Examine for growth, casein hydrolysis and pigment production and/or fluorescence under UV light. Perform an oxidase test on non-pyocyanin producing colonies



Calculate confirmed count for *P. aeruginosa*

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REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Standing Committee of Analysts. Environment Agency. The Microbiology of Drinking Water (2002). Methods for the Examination of Waters and Associated Materials - Part 8: Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa by membrane filtration. London. <http://www.environment-agency.gov.uk/commodata/acrobat/mdwpart8.pdf#search='the%20microbiology%20of%20drinking%20water%20%20part%208'>.
3. The Natural Mineral Water, Spring Water and Bottled Drinking Water (England) Regulations 2007 (Statutory Instrument No.2785). London: HMSO; 2007.
4. PHLS. Hygiene for Hydrotherapy Pools. 2nd ed. London: PHLS; 1999.
5. Health Protection Agency/Health and Safety Executive. Management of Spa Pools - Controlling the Risks of Infection. http://www.hpa.org.uk/publications/2006/spa_pools/spa_pools.pdf.
6. BS EN 12780: 2002. Water quality - Detection and enumeration of pseudomonas aeruginosa by membrane filtration. British Standards Institution (BSI); 2002.
7. Advisory Committee on Dangerous Pathogens. 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
8. Health and Safety Executive, editor. Biological Agents: Managing the risks in laboratories and healthcare premises. 5 A.D.
9. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
10. Control of Substances Hazardous to Health Regulations 2002. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
11. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
12. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.
13. NHS Estates. Health Building Note 15. Facilities for pathology services. 2nd ed. London: The Stationary Office; 2005.
14. BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000.
15. BS 5726: 1992. Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. London: British Standards Institution (BSI); 1992.
16. BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992.

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17. Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. Suffolk: HSE Books; 2001.

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