

HPA STANDARD METHOD

ENUMERATION OF VIABLE MICRO-ORGANISMS: AEROBIC COLONY COUNT BY MEMBRANE FILTRATION METHOD

W 22

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

ENUMERATION OF VIABLE MICRO-ORGANISMS: AEROBIC COLONY COUNT BY MEMBRANE FILTRATION METHOD

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

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Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	W 22
Controlled document title	Enumeration of viable micro-organisms: Aerobic colony count by membrane filtration method

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

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SCOPE OF DOCUMENT

This National Standard Method (NSM) gives general guidelines for the enumeration of viable mesophilic micro-organisms which do not have specialised nutritional requirements after aerobic incubation at 35°C for 72 hours (endoscope washer disinfectant (WD) rinse waters) or 21°C for 7 days (ultrapure dialysis fluid).

If particular micro-organisms are of concern, other recovery conditions (growth medium, incubator temperature etc) should be used as appropriate.

The method should be carried out as an installation, operational, or periodic test for endoscope WDs or ultrapure dialysis fluids when requested by the user.

INTRODUCTION

The Health Technical Memorandum (HTM) 2030² recommends a membrane filtration method for determining the microbial count of endoscope washer disinfectant (WD) rinse waters and for the supply water to WDs. For WDs in which the product is rinsed after the disinfection stage there should be no recovery of micro-organisms from the rinse water.

All other water services supplied to WDs should have less than 100 cfu/100 mL of water. This method is based on section 9 of HTM 2030².

The UK Renal Association also recommends a similar procedure for ultrapure dialysis fluids, but uses incubation times and media suitable for most bacteria found in purified water³. When testing both WD rinse waters and ultrapure dialysis fluid a sample of 100 mL should be filtered using a 0.2 µm pore size membrane filter.

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1 DEFINITIONS

Viable micro-organisms

All aerobic bacteria, yeasts and moulds capable of forming colonies on the medium specified, under the test conditions described.

2 PRINCIPLE

A measured volume of the sample is filtered through a 0.2 µm membrane filter which is then aseptically transferred onto the surface of a Tryptone Soya Agar (TSA) medium for WD rinse waters or Tryptone Glucose Extract Agar (TGEA) for ultrapure dialysis fluids and incubated under the conditions specified.

3 SAFETY CONSIDERATIONS⁴⁻¹⁰

Normal microbiology laboratory precautions apply.

3.1 SAMPLE TRANSPORT AND STORAGE

N/A

3.2 SAMPLE PROCESSING

N/A

4 EQUIPMENT

- Incubator: 35°C ± 2°C
- Incubator: 21°C ± 2°C
- Multibranch filtration manifold with pump
- Sterile disposable or sterile reusable microfunnel filter units
- Sterile filter membranes: cellulose ester 47mm diameter 0.2 µm pore size
- Stainless steel flat ended forceps
- Pyrex vacuum flask: 5 litre

5 CULTURE MEDIA AND REAGENTS

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

Media should be stored at 2°C – 8°C.

Tryptone Soya Agar (TSA)

Tryptone	15.0 g
Soya peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
pH	7.3 ± 0.2

Example Oxoid Ref. Code: CM0131 or use a suitable alternative with the same formulation.

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Tryptone Glucose Extract Agar (TGEA)

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	9.0 g
pH	7.0 ± 0.2

Example Oxoid Ref. Code: CM0127 or use a suitable alternative with the same formulation.

6 SAMPLE PROCESSING

6.1 SAMPLE TRANSPORT AND STORAGE

It is assumed that sampling techniques have been carried out by suitably trained personnel according to HTM 2030² for WD rinse waters or the UK Renal Association guidelines³ for ultrapure dialysis fluids.

6.2 SAMPLE PREPARATION AND DILUTIONS

The nature of the request and condition of the sample should be noted on arrival.

Water samples should be received and handled as described in National Standard Method: W 1 Section 5¹¹.

Samples should be stored and transported at 2°C – 8°C and examined within 4 hours of collection where possible. In exceptional circumstances, if there is a delay, the sample should be stored at 3°C ± 2°C¹² and examined within 24 hours of collection.

6.3 FILTRATION / INCUBATION

Filter 100mL of sample through a sterile membrane filter using sterile disposable or reusable microfunnel filter units.

Use a 0.2 µm filter for both WD rinse waters and ultrapure dialysis fluids.

At the end of filtration close the tap. Use sterile forceps to remove the filter from the filter holder and place grid face up onto the surface of a TSA plate (WD rinse waters) or TGEA plate (ultrapure dialysis fluid).

Incubate at 35°C ± 2°C for 64-72 h (WD rinse waters).

Note: Subject to local agreement plates can be examined at 48 hrs as well as 64-72 hrs and the appropriate staff notified according to local protocol.

Incubate at 21°C ± 2°C for 7 days (ultrapure dialysis fluid).

6.4 COUNTING OF COLONIES

Count and record the number of colonies. Note that very small colonies can be distinguished using a hand lens.

6.5 CONFIRMATORY TESTS

N/A

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7 CALCULATION OF RESULTS

7.1 TEST LIMITS

If there are no colonies on the filter membrane, express the results as 0 colony forming units (cfu) per 100 mL. If there are greater than 100 cfu report as >100 cfu per 100 mL.

8 REPORTING

The test report should specify the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

The result is expressed as the number of cfu per 100 mL.

Upper limits ie >100 are expressed with a comment 'count too high to be estimated at the dilution used'.

Lower limits of <1 are reported as 0/100 mL with an explanatory note stating that '0 = none found in the volume examined'.

8.1 INTERPRETATION OF RESULTS

Report the results using the procedure described in National Standard Method: W 1 Section 9¹¹. Interpretation of positive results should follow QSOP 57 – The microbiological examination of water samples¹³.

Standard comments should be included on the report unless local specifications states otherwise.

For WD rinsewater report as:

"Satisfactory" if there is no recovery of micro-organisms from the rinsewater².

Subject to local agreement with the infection control team counts of 10 or less may be deemed acceptable if the endoscopes are to be used in non-sterile sites.

For Ultrapure dialysis fluid

If the count is <10/100mL "The bacteriological count is satisfactory"³.

9 QUALITY CONTROL

The internal quality control procedures should be carried out following local procedures using the following controls:

9.1 POSITIVE CONTROL

The following procedure should be carried out monthly:

- Prepare a suspension of *E. coli* NCTC 9001 in at least 200mL sterile water containing approximately 30-80 cfu/100mL
- Filter 100 mL in duplicate using the procedures described in 6.3 above. Duplicate results should be within 95% CI¹⁴.

LENTICULE discs are available which will give a consistent count.

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9.2 BLANK CONTROL

A sterility check must be carried out with each batch of test samples or positive controls examined by filtering 100mL of sterile water and proceeding as described in 6.2 and 6.3 above.

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 National Standard Methods (http://www.hpa-standardmethods.org.uk/wg_water.asp). The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

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APPENDIX 1: FLOWCHART SHOWING THE ENUMERATION OF VIABLE MICRO-ORGANISMS: AEROBIC COLONY COUNT BY THE MEMBRANE FILTRATION METHOD

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



Examine within 4 h of collection,
or if this is not possible store at 3°C ± 2°C and examine within 24 h



Mix sample well



Filter 100 mL through a 0.2 µm membrane



WD waters:
Aseptically transfer membrane to
surface of TSA plate



Ultrapure dialysis fluid:
Aseptically transfer membrane to
surface of TGEA plate



Incubate at 35°C ± 2°C for 64-72 h



Incubate at 21°C ± 2°C for 7 days



Count colony forming units (cfu's)



Report cfu/100 mL

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