

NATIONAL STANDARD METHOD

ENUMERATION OF *STAPHYLOCOCCUS AUREUS* BY MEMBRANE FILTRATION

W 10

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division

ENUMERATION OF *STAPHYLOCOCCUS AUREUS* BY MEMBRANE FILTRATION

Issue no: 3.3 Issue date: 03.05.05 Issued by Standards Unit, Evaluations and Standards Laboratory on behalf of the Water Working Group and the Environmental Surveillance Unit, CDSC. Page 1 of 12

Reference no: W 10i3.3

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Suggested citation for this document:

Health Protection Agency (2004). *Enumeration of Staphylococcus aureus by membrane filtration*. National Standard Method W 10 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

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AMENDMENT PROCEDURE

Controlled document reference	W 10
Controlled document title	Standard Operating Procedure for Enumeration of <i>Staphylococcus aureus</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
5/ 03.05.05	3.2	3.3	1	Front page	Redesigned
			2	Status of document	Reworded
			4	Amendment page	Redesigned

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STANDARD OPERATING PROCEDURE FOR THE ENUMERATION OF *STAPHYLOCOCCUS AUREUS* BY MEMBRANE FILTRATION

INTRODUCTION

Scope

The method described is applicable to the enumeration of *Staphylococcus aureus* in samples of swimming pool, hydrotherapy pool and spa pool water.

Background

S. aureus is a component of the normal flora of the nose, skin, respiratory tract and intestinal tract of humans, and is readily shed into water when the body is immersed. It is of concern in the hospital environment because of its potential to cause infections and is also present in other animals. *S. aureus* has also been advocated as an indicator of quality of bathing water, including swimming pools and sea water. *S. aureus* is not normally present in drinking water supplies but detection may be required in food manufacture, pharmaceutical manufacture and in hospitals. No method has been widely accepted for the isolation of *S. aureus* from water.

The method used here is a tentative method and is based on that described in Report 71¹ for the enumeration of staphylococci.

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1.0 DEFINITIONS

Staphylococcus aureus

Microorganisms which form typical colonies on a membrane placed on the selective medium described in this method and which show positive reactions on DNase and coagulase or commercial equivalent.

2.0 PRINCIPLE

The test volume of water is filtered and the membrane is placed onto mannitol salt agar modified by the addition of 0.005% sodium azide (5MSA). This is the medium M-5LSMA of Stengren and Starzyk² modified by the omission of the egg yolk. The medium contains sodium chloride (7.5%) and sodium azide (0.005%) which together allow the selection of staphylococci whilst suppressing micrococci and Gram positive bacilli. It also contains mannitol as the fermentable carbohydrate and phenol red as the indicator of acid production. Incubation at 30°C for 42 - 44 hours allows yellow pigmentation typical of *S. aureus* to develop.

3.0 SAFETY CONSIDERATIONS³⁻¹²

Normal microbiology laboratory precautions apply.

3.1 Specimen collection

N/A

3.2 Specimen transport and storage

Compliance with current postal and transportation regulations is essential.

3.3 Specimen processing

- Care must be taken when removing objects from boiling water after disinfection
- Sodium azide is used in this method as a component of the medium. This compound is highly toxic if ingested or inhaled and care must be taken when handling it. Solutions containing azide should not be discharged through metal pipework since they can form spontaneously explosive compounds. Azides can be decomposed by treatment with an excess of nitrite solution. However, as the medium contains only a low concentration of azide and has a relatively low toxicity, it is safe to handle and dispose of in the normal manner in the laboratory

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

4.0 EQUIPMENT

Usual laboratory equipment and in addition:

- Membrane filtration manifold
- Filter funnels graduated to 50 mL and 100 mL
- Pyrex vacuum flask with protective jacket or equivalent: large volume e.g 5 l
- Vacuum pump with moisture trap or protective filter, or alternative vacuum source

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- Incubators: 30°C ± 1°C
- 37°C ± 1°C
- Stainless steel flat tipped forceps
- Boiling waterbath (instrument steriliser)
- Petri dishes
- Cellulose ester 0.45 µm pore size grid filters
- Automatic pipettors with associated sterile pipette tips capable of delivering up to 10 mL and 1 mL volumes (optional)
- Pipettes (sterile total delivery) 10 mL and 1 mL graduated in 0.1 mL volumes (optional)

5.0 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	

Quarter strength Ringer's solution

Sodium chloride	2.25 g
Potassium chloride	0.11 g
Calcium chloride, anhydrous	0.12 g
Sodium bicarbonate	50 mg
Water	1 L

Mannitol salt agar and 0.005% sodium azide

Beef extract	1.0 g
Peptone	10.0 g
D(-) mannitol	10.0 g
Sodium chloride	75.0 g
Sodium azide	50 mg
Phenol red	25 mg
Agar	15.0 g
Water	1 L
pH 7.4 ± 0.2 at 25°C	

Blood Agar

Columbia agar or any other suitable base with 5% horse blood

DNase Agar

Tryptose	20.0 g
Deoxyribonucleic acid	2.0 g
Sodium chloride	5.0 g
Agar	12.0 g
Water	1 L
pH 7.3 ± 0.2 at 25°C	

Hydrochloric acid (1N)

or

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Toluidine blue solution

Toluidine blue	0.1 g
Water	100 mL

Coagulase reagent

Rabbit plasma or any commercial alternative proven to be comparable to the rabbit plasma.

6.0 SAMPLE PROCESSING

6.1 Sample Preparation

Water samples should be received and handled as described in SOP W1 Section 5. In brief the nature of the request and condition of the specimen should be noted on arrival and the specimen stored at 2°C -10°C. Samples should be analysed 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 SOP W1 Section 5 select suitable volumes for analysis and prepare any necessary dilutions.

6.2 Filtration and Incubation

Following the procedures laid down in SOP W1 Section 5 filter a measured volume of sample through the membrane.

For potable quality waters use volumes of 100 mL. Place the membrane onto mannitol salt agar with 0.005% sodium azide (5MSA) and place in an incubator at 30°C ± 1°C for 48 hours ± 2 hours.

6.3 Counting of colonies

After incubation, enumerate the presumptive *S. aureus* by counting all colonies that are off-white through cream to yellow in colour.

6.4 Confirmatory tests

Select colonies for confirmation as described in SOP W1 Section 5 for confirmatory testing by DNase and coagulase production. Inoculate each colony onto a DNase agar plate and plate out onto a segment of a blood agar plate. Transfer the plates to an incubator at 37°C for 18-24 hours.

DNase production

Flood the DNase plate with toluidine blue solution (TBS) or normal hydrochloric acid (HCl). After about 30 seconds, discard the excess reagent (TBS or HCl) into a chemical waste container.

Positive reactions are as follows:

Toluidine blue solution: colonies surrounded by a pink zone against a blue background.

Hydrochloric acid: colonies showing a defined zone of clearing.

Coagulase production

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Using the growth on blood agar (BA), perform a slide coagulase test on the strains giving a positive DNase test. Allow the rabbit plasma to equilibrate at room temperature (10-15 minutes) before use and perform the test as follows:

Place two drops of water on a microscope slide and make a creamy suspension in each drop from a colony on BA. Mix a loopful of undiluted coagulase reagent into one of the suspensions. The presence of bound coagulase is demonstrated by the appearance of microscopic clumping within 5-10 seconds and the absence of autoagglutination in the second suspension.

For commercially produced test kits follow the manufacturers instructions.

Colony types are confirmed as *S. aureus* if they show typical colonial morphology on blood agar and give positive reactions in the DNase and coagulase tests.

7.0 CALCULATION OF RESULTS

Calculate the presumptive count of the test organisms as follows:

Presumptive count / 100 mL =

$$\frac{\text{Number of colonies counted}}{\text{Volume tested}} \times 100$$

Following the procedures described in SOP W1 Section 7, calculate the number of confirmed *S. aureus* detected in the original sample.

8.0 REPORTING

Report the results using the procedure described in SOP W1 Section 9.

If *S. aureus* are not detected then report as:

'Not detected per 100 mL'

If the test organisms are detected report as:

'a per 100 mL'

where **a** is the confirmed count.

9.0 QUALITY CONTROL

Membrane filtration

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

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

Positive control

Prepare a suspension of *S. aureus* NCTC 6571

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Negative control

Prepare a suspension of *Escherichia coli* NCTC 9001

Blank control

Filter 1 L of sterile distilled water, peptone saline diluent or quarter strength Ringer's solution using the same funnel as that used for the positive control following sterilisation. Incubate all tests and perform all procedures in parallel with the routine test samples.

Confirmatory test

The DNase plate must be inoculated with *S. aureus* NCTC 6571 as the positive control and *S. epidermidis* NCTC 11047 as the negative control.

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Flowchart showing the process for the enumeration of *Staphylococcus aureus* by membrane filtration

Transport to laboratory at 2°C –10°C out of direct sunlight in a suitable container



Store at 2°C – 10°C in the dark and analyse as soon as is practicable on the day of collection, otherwise within 24 hours of collection



Mix sample well and make any necessary dilutions



Filter



Place the membrane onto mannitol salt agar with 0.005% sodium azide (5MSA)



Incubate at 30°C for 48 hours



Count colonies which are off white through cream to yellow in colour.



Subculture to DNase agar and blood agar and incubate at 37 °C for 18-24 hours



Identify *S. aureus* colonies using slide coagulase and DNase tests



Calculate the confirmed count for *S. aureus* count

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REFERENCES

- 1 Standing Committee of Analysts. The Microbiology of Water 1994 - Part 1 - Drinking Water. Reports on Public Health and Medical Subjects No 71: Methods for the Examination of Waters and Associated Materials .London; 2002. See http://www.environment-agency.gov.uk/science/219094/399393/401849/?lang=_e®ion=&projectstatus=&theme=&subject=&searchfor=SCA&topic=&area=&month
- 2 Stengren SR, Starzyk MJ. A modified medium for the recovery of *Staphylococcus* from water. Microbios 1984;41:191–203
- 3 Advisory Committee on Dangerous Pathogens. Categorisation of biological agents according to hazard and categories of containment, 4th ed. Suffolk: HSE Books; 1995 (with supplements 1, 1998 and 2, 2000)
- 4 Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety precautions: notes for guidance, 4th ed. London: Public Health Laboratory Service (PHLS); 1993
- 5 Control of Substances Hazardous to Health Regulations 2002. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002
- 6 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; April 2002
- 7 Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; March 2002
- 8 NHS Estates. Health Building Note 15. Accommodation for pathology services. 1st ed. London: Her Majesty's Stationary Office (HMSO); 1991 (Out of print – 2nd edition in press)
- 9 BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000
- 10 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
- 11 BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992
- 12 Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. Suffolk: HSE Books; 2001

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