

STANDARDS AUSTRALIA/STANDARDS NEW ZEALAND

RECONFIRMATION

OF

AS/NZS 4276.20:2003

Water microbiology

**Method 20: Examination for coagulase positive staphylococci, including
Staphylococcus aureus, by membrane filtration**

RECONFIRMATION NOTICE

Technical Committee FT-020 has reviewed the content of this publication and in accordance with Standards Australia procedures for reconfirmation, it has been determined that the publication is still valid and does not require change.

Certain documents referenced in the publication may have been amended since the original date of publication. Users are advised to ensure that they are using the latest versions of such documents as appropriate, unless advised otherwise in this Reconfirmation Notice.

Approved for reconfirmation in accordance with Standards Australia procedures for reconfirmation on 22 November 2013.

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NOTES

Australian/New Zealand Standard™

AS/NZS 4276.20

Water microbiology

Method 20: Examination for coagulase positive staphylococci, including *Staphylococcus aureus*, by membrane filtration

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee, FT-020, Water Microbiology, as part of a series of methods for the microbiological examination of waters for domestic and industrial use.

Staphylococci, particularly coagulase positive staphylococci and *Staphylococcus aureus*, are of concern in the hospital environment because of their potential to cause infections. They have also been advocated as indicators of the quality of bathing water including swimming pools, hydrotherapy pools and sea water. They are not normally present in drinking water supplies but their detection may be required in food and pharmaceutical manufacture and in hospitals.

Members of the genus *Staphylococcus* are gram-positive cocci, facultatively anaerobic, non-motile, do not form spores and are usually catalase positive and sensitive to lysostaphin. In the context of this method, coagulase positive staphylococci are defined as producing typical colonies on Baird-Parker agar and a positive result in the coagulase test. *Staphylococcus aureus* is a coagulase positive staphylococcus that can be identified by verifying certain phenotypic characteristics, including biochemical reactions and cell wall components.

The term 'informative' has been used in this Standard to define the application of the appendix to which it applies. An 'informative' appendix is only for information and guidance.

METHOD

1 SCOPE

This Standard sets out a method for detecting coagulase positive staphylococci and *Staphylococcus aureus* (*S.aureus*) in water by the membrane filtration method.

NOTES:

- 1 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.
- 2 The optimum range for membrane filter counts is 20-80 colonies. If the confirmed total count from one or more membranes is less than 20, then the derived sample count, which is an estimate of the true count in the sample (population parameter), is regarded as having a low precision. At the other extreme, when membranes containing greater than 80 colonies are counted, the counting error tends to be large. Again, the derived counts are regarded as having low precision. Derived counts of low precision should be indicated on reports by flagging them as 'estimated' or 'approximate'.
- 3 A flow diagram of the procedure is shown in Appendix A.



2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

4276 Water microbiology

4276.1 Method 1: General information and procedures

4276.2 Method 2: Culture media, diluents and reagents

3 PRINCIPLE

The test volume of sample is filtered through a membrane filter, retaining the bacteria in the sample on or near the surface of the membrane. The membrane is placed onto the surface of an agar medium containing the selective agents glycine, lithium, and tellurite, and egg yolk emulsion as a differential ingredient. Plates are incubated aerobically for the specified time and temperature. Typical colonies are counted and recorded as presumptive counts. A proportion of presumptive colonies is confirmed as coagulase positive staphylococci by testing for tellurite reduction in conjunction with proteolytic and lipase activity, followed by the ability to coagulate plasma or give a positive reaction in an equivalent commercially available test kit. *Staphylococcus aureus* can be identified by using conventional tests or by using commercially available kits.

4 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES

4.1 Culture media

4.1.1 Baird-Parker agar (BP)

4.1.2 Brain heart infusion (BHI) broth

NOTE: Formulations and procedures for preparing some culture media are given in Appendix B.

4.1.3 Nutrient agar

4.2 Reagents (see AS 4276.2)

4.2.1 Saline solution, 0.85%

4.2.2 Rabbit blood plasma—oxalated or heparinized but not citrated. When purchased in freeze-dried form, prepare as directed by the manufacturer.

4.3 Reference cultures

4.3.1 Positive culture

Staphylococcus aureus ACM 556, NCTC 6571, ATCC 9144, NZRM 87—coagulase positive

4.3.2 Negative culture

Staphylococcus epidermidis ACM 3978, NCTC 6513—coagulase negative.

NOTE: NZRM does not contain an equivalent culture for NCTC 6513.

4.3.3 Use of reference cultures

When testing a sample of water by this standard method, cultures of the positive (4.3.1) and negative (4.3.2) reference cultures shall be submitted to the test procedures at the same time to demonstrate and ensure that typical positive and negative growth characteristics and test reactions are exhibited by the respective reference microorganisms.

NOTES:

- 1 ACM—Australian Collection of Microorganisms
NCTC—National Collection of Type Cultures
ATCC—American Type Culture Collection
NZRM—New Zealand Reference Culture Collection (Medical Section)
- 2 These cultures are obtainable from the following Laboratories:

In Australia:

Australian Collection of Microorganisms (ACM)
Department of Microbiology
The University of Queensland
BRISBANE QLD 4072

In New Zealand:

New Zealand Reference Culture Collection (Medical Section) (NZRM)
ESR Kenepuru Science Centre
Kenepuru Drive
Porirua

5 SAMPLES**5.1 Laboratory samples**

Samples shall be prepared as set out in AS 4276.1.

5.2 Dilutions

Dilutions of the laboratory sample shall be prepared as required, using the procedure described in AS 4276.1.

6 PROCEDURE**6.1 Membrane filtration**

The procedure for membrane filtration shall be as follows:

- (a) After preparing the sample and making any required dilutions as described in AS 4276.1, filter the test volume as in membrane filtration method, through a membrane of 0.45 µm average porosity.
- (b) Roll each membrane onto the surface of a BP agar plate, taking care to avoid trapping air bubbles beneath the membrane.
- (c) Incubate the plates aerobically at $36 \pm 1^\circ\text{C}$ for 48 ± 2 h.

6.2 Presumptive identification of coagulase positive staphylococci

The procedure shall be as follows:

- (a) Presumptive colonies of coagulase positive staphylococci are smooth, entire and range in colour from slate grey to jet-black.
- (b) Count and record each distinct colony type. The total is the presumptive count of coagulase positive staphylococci.

6.3 Confirmation of coagulase positive staphylococci

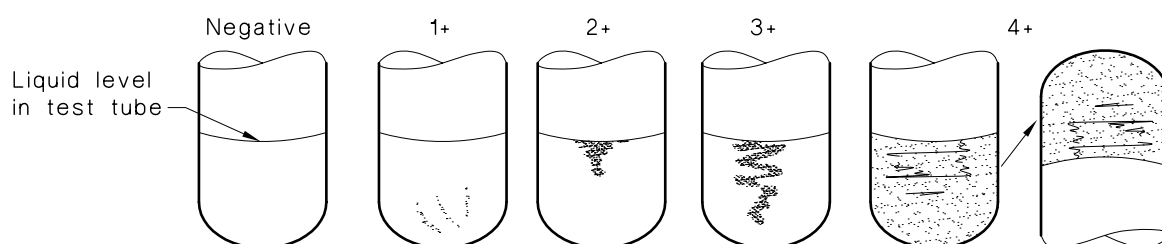
The procedure shall be as follows:

- (a) Select a representative number of discrete colonies from each morphological group of presumptive organisms for confirmation. The number from each group shall approximate the square root of the count of that group up to a maximum of 10.

- (b) Subculture each selected colony onto a BP agar plate divided into sectors and incubate the plates aerobically at $36 \pm 1^\circ\text{C}$ for 48 ± 2 h.
- (c) Examine the plates for growth typical of coagulase positive staphylococci. Typically, tellurite is reduced to produce growth that ranges in colour from slate grey to jet-black, surrounded by clear zones due to proteolytic activity. This clear zone with typical grey-black growth is diagnostic of coagulase positive staphylococci. Most strains also produce opaque haloes around colonies due to the action of lipase.

NOTE: Some strains of coagulase positive staphylococci do not produce an egg yolk reaction. Growth of presumptive coagulase positive staphylococci but without an egg yolk reaction should be tested further.

- (d) The tube coagulase test detects 'free' coagulase. Perform the test on cultures showing growth typical of coagulase positive staphylococci. The tube coagulase test can be performed in a number of different ways. The following details one of these:
 - (i) Inoculate 5 mL to 10 mL volumes of BHI broth with the cultures.
 - (ii) Incubate the broths at $36 \pm 1^\circ\text{C}$ for 20 ± 2 h.
 - (iii) Prepare a 1/10 dilution of the blood plasma using the saline solution or as otherwise recommended by the manufacturer.
 - (iv) Dispense to each tube 0.5 mL of the prepared plasma into each of the required number of test and control tubes, of size approximately $10\text{-}12\text{ mm} \times 75\text{ mm}$.
 - (v) Add to each tube 0.1 mL of the BHI broth culture from Step (ii) above.
- NOTE: If using commercial plasma, follow the manufacturer's instructions regarding the volumes of plasma and BHI broth to be used.
- (vi) Incubate the tubes at $36 \pm 1^\circ\text{C}$.
 - (vii) Examine the tubes after 1, 3 and 6 h incubation and, if not positive, again after leaving overnight at room temperature. The formation of a distinct clot giving a 3+ or 4+ reaction (see Figure 1) is evidence of coagulase production. Small or poorly organized clots (1+ and 2+) are further tested with the slide coagulase test.



Negative — no evidence of fibrin formation

1+ — small unorganized clot

2+ — small organized clot

3+ — large organized clot

4+ — entire content of tube coagulates and is not displaced when tube is inverted

FIGURE 1 GUIDE FOR ASSESSING COAGULASE TEST REACTIONS

- (e) The slide coagulase test detects clumping factor or 'bound' coagulase. The test can be performed in a number of different ways. The following details one of these:
 - (i) Subculture the BHI broth cultures and control cultures onto a nutrient agar plate and streak for isolated colonies. Incubate overnight at $36 \pm 1^\circ\text{C}$.