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Water microbiology

Method 3: Enumeration of heterotrophic microorganisms – Pour plate, spread plate, membrane filtration and most probable number techniques

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Originated as AS 4276.3.1—1995 and AS/NZS 4276.3.2:2003.
AS 4276.3.1—1995 revised and redesignated as AS/NZS 4276.3.1:2007.
Revised and redesignated as AS 4276.3:2021.

Preface

This Standard was prepared by the Standards Australia Committee, FT-020, Water Microbiology, to supersede AS/NZS 4276.3.1—2007, *Water microbiology, Method 3.1: Heterotrophic colony count methods — Pour plate method using yeast extract agar,* and AS/NZS 4276.3.2—2003, *Water microbiology, Method 3.2: Heterotrophic colony count methods — Plate count of water containing biocides*.

The objective of this document is to establish a standard method for the examination of water, with or without biocides, for the enumeration of heterotrophic microorganisms that covers a choice of possible techniques. The choice of procedure and medium will depend on the sample to be tested and the application of the results.

The major changes in this edition are as follows:

(a) Combine the methods of AS/NZS 4276.3.1—2007 with AS/NZS 4276.3.2—2003.

(b) Update culture media and reagents.

(c) Update reference cultures.

(d) Update the normative and informative references.

The term "informative" is used in Standards to define the application of the appendices to which it applies. An "informative" appendix is only for information and guidance.

Contents

Introduction

Waters of all kinds contain a variety of microorganisms derived from various sources and estimation of their overall number can provide useful information for assessing and monitoring water quality. Microorganisms which are able to survive in water usually grow better in the laboratory at about 22 °C ± 2 °C than at higher temperatures, the results often reflecting the environmental and seasonal conditions prevailing at the time. In contrast, microorganisms which grow well at 36 °C ± 2 °C generally survive with difficulty in water.

Heterotrophic colony counts are useful for assessing the integrity of ground water and the effectiveness of water treatment processes such as coagulation, filtration and disinfection and provide an indication of the cleanliness and integrity of the distribution system. They can also be used to assess the suitability of a supply for the preparation of food and drink, where the water supply should contain few microorganisms to avoid contaminating the product with spoilage organisms.

For these reasons, separate colony counts may be performed at 22 °C ± 2 °C and/or at 36 °C ± 2 °C. For cooling tower waters, plate counts are incubated at 36 °C ± 2 °C.

Waters which may contain biocides, e.g. cooling tower waters, require a modification of procedures to eliminate possible interference with counts.

In samples of water where an unidentified biocide may be present or where effective neutralization is not available it is necessary to use dilution of the sample to minimize interference by the biocide with counts. This will increase the lower detection limit.

With regard to *Legionella* control legislation in most states and territories, the heterotrophic colony count (HCC) is used as a surrogate measure for the cleanliness of cooling towers, and the procedure of choice is listed in AS/NZS 3666.3, *Air-handling and water systems of buildings — Microbial control, Part 3: Performance-based maintenance of cooling water systems*.

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. They include bacteria and fungi. The heterotrophic count (HC) refers to the number of organisms grown in non-specific culture media without inhibitory or selective agents. Even though the test is non-selective, there will be a proportion of the microorganisms present in the water sample that may not be recovered. The types of organisms detected by HC tests can vary widely depending on the source of water, and time of year. It is important to note that the heterotrophic count results may differ for each of the procedures described below due to different incubation temperatures and times, the methods/techniques used and, to a lesser extent, differences in the media formulation. This means that equivalence of results derived from a single sample using different techniques and procedures is not to be expected.

Where HC limits exist in standards, or are referred to in guidelines, it is essential to prescribe and adhere to the technique, medium and incubation conditions where these have been prescribed.

Where there are no limits for HC set out in water standards or guidelines, the absolute HC result is not relevant. The main purpose of HC is in detecting changes in the cleanliness of a water system. Consequently, it is important not to change the technique when looking for temporal or locational changes within a water system.

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1 Scope and general

1.1 Scope

This Standard specifies methods for enumeration of heterotrophic microorganisms in water using the following techniques:

(a) Pour plate.

(b) Spread plate.

(c) Spiral plate.

(d) Membrane filtration.

(e) Most probable number (MPN).

These techniques are intended to measure the operational effectiveness of treatment processes and be used for the general examination of many types of water to evaluate and/or note changes in water quality.

This document does not cover the comparison of results obtained from different techniques.

1.2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document:

NOTE Documents for informative purposes are listed in the Bibliography.

AS 2031, *Water quality — Sampling for microbiological analysis (ISO 19458:2006, MOD)*

AS 5140, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014, MOD)*

AS 4276.1, *Water microbiology, Method 1: Water quality — General requirements and guidance for microbiological examinations by culture (ISO 8199:2018, MOD)*

1.3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

1.3.1
count
observed number of objects, such as colonies, determined by direct counting, or most probable number (MPN) estimation based on statistical calculation using the number of positive units in a dilution series of a test sample

1.3.2
may
indicates the existence of an option