International Standard 7704

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Water quality — Evaluation of membrane filters used for microbiological analyses

Qualité de l'eau — Évaluation des membranes filtrantes utilisées pour des analyses microbiologiques

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

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Water quality — Evaluation of membrane filters used for microbiological analyses

0 Introduction

Many membrane filter comparison studies which have been reported in the literature indicate that there are significant differences between various chemical compositions, brands and batches of membranes in their ability to recover bacteria from water samples.

Thus, it is very important that one of the basic tools of aquatic microbiology, the membrane filter, be standardized as much as possible, not only to provide consistent results, but also to enable the development of standardized procedures for enumerating specific micro-organisms.

1 Scope

1.1 This International Standard specifies a method for the evaluation and comparison of water-testing membrane filters intended for the enumeration of specific organisms and mixed microbial populations.

1.2 The method provides general guidelines for comparative testing of the recoveries of bacteria, yeasts and other fungi on membrane filters, as compared to recoveries by the spread plate and pour plate techniques.

2 Field of application

2.1 This method is applicable to the user's evaluation of any microporous filter intended for use with aquatic samples. Its range covers any pore size filter which may be useful in a specific application.

2.2 For specific applications, it is expected that suitable media, incubation temperature, incubation duration, incubation atmosphere and controls (spread or pour plate) will be used. Results obtained from one species or group of micro-organisms may not be valid for other groups.

3 Definition

For the purpose of this International Standard, the following definition applies.

membrane filter : A thin non-fibrous filtration medium for liquids and gases, having a mean pore size larger than 0,01 µm in diameter, on which particles larger than the rated pore size are retained at or near the delivery surface when suction or pressure is applied.

4 Principle

4.1 Filtration of aqueous or pure cultures in liquid suspension through test membrane filters, using conventional procedures. Five replicates are minimum sample requirements; a total of 200 colonies is considered the minimum number for statistical comparison.

4.2 Evaluation of the efficiency of each type of membrane filter by

a) counts obtained on non-selective medium using spread or pour plate technique versus membrane filtration technique counts on the same medium (experience indicates that under these conditions the best membrane filter counts are 80 to 90 % of those obtained by plate counts) ;

b) results for specific organisms obtained on selective membrane filter medium using spread or pour plate techniques versus membrane filtration technique on the same medium.

NOTE — Pour plate control may provide fewer colonies than spread plate control.

5 Diluent, culture media and reagent

5.1 Basic material

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluents and culture media, dehydrated basic components or complete dehydrated media be used. The manufacturer's instructions shall be rigorously followed.

The chemical products used for the preparation of the culture media and the reagents shall be of recognized analytical quality.

The water used shall be distilled or deionized water, free from substances that might inhibit the growth of micro-organisms under the test conditions.

Measurements of pH shall be made using a pH meter, measurements being referred to room temperature.

If the prepared culture media are not used immediately, they shall, unless otherwise stated, be stored in the dark at 4 ± 2 °C, for no longer than 1 month, in conditions which do not produce any change in their composition.

5.2 Diluent

Any appropriate sterile diluent may be used. Use of peptone [0,1 % (m/m)] water has been found to be suitable. This minimizes the shock to the organisms of pure water.

5.3 Agar media

5.3.1 Non-selective medium.

Tryptone soya agar or a similar medium may be used as the non-selective medium in this test.

Prepare the medium and dispense a measured amount of medium into Petri dishes (in the case of membrane filter counts and spread plate counts) or into suitable tubes (in the case of pour plate counts). The depth of agar in the Petri dishes should be at least 3 mm. All media used for each test should be prepared from the same batch and prepared at the same time.

5.3.2 Selective medium.

The media used should be appropriate for the organisms being used, either in mixed culture or pure culture.

Prepare the medium and dispense a measured amount of medium into Petri dishes (in the case of membrane filter counts and spread plate counts) or into suitable tubes (in the case of pour plate counts). The depth of agar in the Petri dishes should be at least 3 mm. All media used for each test should be prepared from the same batch and prepared at the same time.

6 Apparatus and glassware

Clean all glassware and filtration equipment thoroughly, using a suitable detergent in hot water, rinse with hot water, and then rinse with distilled water.

Follow standard microbiological laboratory practices for preparing glassware and filtration equipment prior to sterilization in the autoclave. Autoclave at 121 °C for 20 min for wet sterilization or for dry sterilization heat at 170 °C for at least 60 min (6.7).

Usual microbiological and laboratory equipment and

6.1 Filtration units, for membrane filters, with vacuum flask tubing, moisture trap flask, and connectable to a vacuum source.

6.2 Vortex mixer, or similar mixer to mix cultures for testing (optional).

6.3 Forceps, with flat, non-serrated tips.

6.4 Incubator, water-bath or **heat sink** capable of being maintained at a variety of temperatures.

The appropriate incubator should be frequently checked to ensure that it is capable of maintaining the required temperature for at least 24 h. A thermometer that has been checked for accuracy should be placed in the incubator on the shelf where the plates will be incubated.

6.5 Colony counting apparatus, with suitable illumination and magnification.

6.6 Hand tally counter.

6.7 Autoclave, or other sterilizing equipment.

6.8 Turntable (optional) and **glass spreading rod.**

6.9 Sterile vented Petri dishes, appropriate sizes.

6.10 Sterile calibrated pipettes, of capacities 0,1; 1,0; and 10 ml.

7 Preparation of cultures for testing

7.1 Whether natural water, effluent samples or pure cultures are being used to evaluate the membranes, they should be analysed prior to the test in order to obtain the dilution to be used (9.2.1). At all stages mix the samples or cultures thoroughly (6.2) to obtain homogeneous distribution.

7.2 If pure cultures in stressed or unstressed state are used, establish the concentration of the test organism on an appropriate medium to ensure that the correct dilution factor is used to obtain the proper counting range (9.2.1).

7.3 The samples used to establish proper dilutions and counting ranges may be used in the formal test if refrigerated immediately after testing and not held for an excessively long period (maximum 48 h). Mix samples thoroughly to obtain maximum homogeneity.

8 Membrane filters

The membrane filters shall be sterile.

9 Procedure

9.1 Inoculation and incubation

Prior to conducting the test, aseptically dry the Petri dishes (6.9) containing the nutrient non-selective agar (5.3.1) or the agar specific for the test organism (5.3.2), in accordance with one of the following methods.

a) With covers on, store the Petri dishes inverted in the dark if the media are light sensitive at 25 to 30 °C for 15 to 17 h.

b) With covers on, store the Petri dishes inverted in the dark at 45 to 50 °C for 2 to 3 h.

c) With covers removed, place the Petri dishes in a filtered air laminar flow hood at room temperature for 1 h. If this procedure is chosen, sterility controls shall be used.