International Standard

ISO 7704

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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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.

Cultures for testing (see clause 7) should be readily available for conducting the tests without delay. The same cultures shall be used to inoculate the different media. Equal volumes, of between 0,1 to 0,5 ml of an appropriate dilution of the culture, should be used to inoculate control plates or for filtration through membranes. The volume 0,5 ml shall not be exceeded because of spread plate requirements.

To avoid test bias, spread plate controls should be carried out alternatively with the membrane filter test, using the same nutrient or selective media as the membrane filter test.

If more than one brand of membrane filter is being compared, the membranes should be alternated with control spread or pour plates until the required number of replicates has been made with the specific culture.

The media with and without membrane filters are incubated after seeding under temperature and time conditions appropriate to the organisms being studied.

9.1.1 Membrane filter cultures

9.1.1.1 With sterile forceps (6.3), aseptically remove the membrane filter (see clause 8) to be tested and centre the membrane grid side up or face-up on the filter holder base. Place a filter funnel on to the assembly and secure as required by the specific holder. Connect the filtration flask and vacuum trap to a vacuum source (6.1).

9.1.1.2 With vacuum off, add 20 to 30 ml of sterile dilution water (5.2). Aseptically transfer (6.10) the same volume from the same well-mixed sample dilution that was used in the spread plate procedure (9.1.1.1), into the dilution water in the funnel. Apply the vacuum and filter the entire contents. Rinse the funnel with 20 to 30 ml of sterile dilution water twice, applying the vacuum continuously. Turn off the vacuum immediately after the last rinse has passed through the filter. Remove the filter funnel and with sterile forceps remove the membrane filter from the base.

9.1.1.3 Place the test membrane on either the non-selective medium (5.3.1) or the selective medium (5.3.2), depending on the rotation order. Roll the membrane filter on to the agar surface in the Petri dish, making sure that air is not entrapped between the membrane and the agar surface. If an air bubble is observed, the membrane should be raised and again rolled on to the agar to eliminate the air.

9.1.1.4 All plates should be incubated (6.4) under temperature and time conditions appropriate to the organisms being studied.

9.1.2 Spread plate controls

9.1.2.1 Aseptically deliver (see 6.10) and spread [using a glass spreader (6.8) that has been dipped in ethanol and flamed] 0,1 to 0,5 ml of the appropriate dilution of the culture on to the predried surface of either the nutrient non-selective agar or the membrane filter selective agar. To aid even distribution in the inoculum, plates can be put on a turntable (6.8). Replace Petri dish covers and allow the aliquot of culture to be completely absorbed before inverting.

9.1.2.2 All plates should be incubated (6.4) as soon as possible after the moisture is absorbed, under temperature and time conditions appropriate to the organisms being studied.

9.1.3 Pour plate controls

9.1.3.1 Aseptically deliver (6.10) the same volume from the same well-mixed sample dilution that was used in the membrane filtration procedure (9.1.2.1) into the bottom of a sterile Petri dish (6.9). To this add one standard portion of melted agar (45 °C), either non-selective nutrient agar (5.3.1) or membrane filter selective agar (5.3.2) and thoroughly mix the two solutions by back and forth and circular motions of the dish.

9.1.3.2 Allow the agar to solidify thoroughly, then invert the dish and incubate (6.4) for the required period at the required temperature.

9.1.3.3 For sterility control of the agar, prepare plates from several tubes of melted agar.

9.2 Interpretation

9.2.1 Membrane filter cultures

Use a colony counting apparatus (6.5) to count all typical colonies (mixed or pure cultures) on the surface of the membrane. If more than one dilution of a culture was employed for the test, the dilution selected for counting should have a target colony count between 25 and 100 and a sufficient number of replicates to give at least 200 colonies per treatment.

9.2.2 Spread plate and pour plate

Use the same culture dilution, selected for the membrane count for the spread plate and pour plate counts for recovery comparisons. Use a colony counting apparatus to help count (6.6) all surface and subsurface colonies. Follow routine counting procedures as established in the tester's laboratory.

10 Expression of results

10.1 Calculation

For each culture used for testing, calculate the arithmetic mean of the counts of the target organism from the five (or more) replicates of each treatment.

The recovery, R, expressed as a percentage, for each culture, is given by the equation

$$R = \frac{m_{\mathrm{m}}}{m_{\mathrm{c}}} \times 100$$

where

m_{m} is the mean of the membrane filter counts;

m_{c} is the mean of the pour plate or spread plate counts.

3

10.1.1 Establish the percentage recovery from the data obtained with membrane filter count and plate count on non-selective agar.

10.1.2 Establish the percentage recovery from the data obtained with membrane filter count and plate count on selective agar.

10.2 Interpretation of results

10.2.1 When testing batch to batch variation in membrane filters produced by one manufacturer, to be considered equivalent, replicate counts should be within the 95 % confidence interval of the batch with which they are being compared.

The 95 % confidence interval is obtained by applying the following formulae :

a) for upper limit counts, $m + 2(\sqrt{m+1} + 1)$;

b) for lower limit counts, $m - 2(\sqrt{m+1} - 1)$;

where m is the arithmetic mean of the bacterial counts on the batch of control membrane filters.

10.2.2 When comparing membrane filter counts versus control plating counts (noting that spread plate procedures may produce the higher plating counts, thus the spread plate procedure is recommended), membranes producing counts 80 % or more of the control plating counts will be considered acceptable. If a membrane is to be used solely for the enumeration of a specific organism, then selective media data are used.

10.2.3 Consider a particular brand or type of membrane preferable only if it yields mean colony counts, m_m, which are 20 % or more in excess of those on other membranes.

11 Test report

The test report shall show the method used and the results obtained, indicating clearly the method of expression used. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the membrane filters, cultures, media and diluents used.