SLOVENSKI STANDARD
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Kakovost vode - Ugotavljanje prisotnosti in števila fekalnih streptokokov - 2. del: Metoda membranske filtracije

Water quality -- Detection and enumeration of faecal streptococci -- Part 2: Method by membrane filtration

Qualité de l'eau -- Recherche et dénombrement des streptocoques fécaux -- Partie 2: Méthode par filtration sur membrane

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International Standard 7899/2

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Water quality — Detection and enumeration of faecal streptococci — Part 2: Method by membrane filtration

Qualité de l'eau — Recherche et dénombrement des streptocoques fécaux — Partie 2: Méthode par filtration sur membrane

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

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.

International Standard ISO 7899/2 was prepared by Technical Committee ISO/TC 147, *Water quality*.

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Water quality — Detection and enumeration of faecal streptococci — Part 2: Method by membrane filtration

0 Introduction

Worldwide, opinions differ as to which streptococci should be regarded as indicators of faecal pollution. In this part of ISO 7899, a method is described for the isolation of streptococci possessing the group D antigen. For water examination purposes these organisms can be regarded as indicators of faecal pollution. It must be realized, however, that small numbers of group D streptococci found in water could also originate from other habitats. The selectivity of the media used is such that a reliable count of group D streptococci will be obtained for most practical purposes. Because serological confirmation is not included, the term "faecal streptococci" is used. In special circumstances, however, further identification and serological grouping of the streptococci isolated may be required.

ISO 7899 consists of the following parts:

Part 1: Method by enrichment in a liquid medium.

Part 2: Method by membrane filtration.

1 Scope

This part of ISO 7899 specifies a method for the detection and enumeration of faecal streptococci in water by membrane filtration.

2 Field of application

The method can be applied to all types of water, except when a large amount of substance liable to be retained by the membrane is present.

3 References

ISO 5667, *Water quality — Sampling —*

Part 1: Guidance on the design of sampling programmes.

Part 2: Guidance on sampling techniques.

Part 3: Guidance on the preservation and handling of samples.

ISO 7704, *Water quality — Evaluation of membrane filters used for microbiological analyses.*

4 Definitions

4.1 presumptive faecal streptococci: Bacteria that give a positive reaction with the media (6.2.1 and 6.2.2) specified in this part of ISO 7899.

4.2 faecal streptococci: Bacteria that give a positive reaction with the medium (6.2.3) specified in this part of ISO 7899 and that give a negative reaction in the catalase test.

5 Principle and reactions

5.1 Filtration, incubation and enumeration

The enumeration of faecal streptococci is based on filtration of a specified volume of water sample through a membrane filter with a pore size (0,45 µm) sufficient to retain the bacteria. The filter is placed on a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and 2,3,5-triphenyltetrazolium chloride, a colourless dye, that is reduced to red formazan by faecal streptococci.

After incubation all raised colonies which show a red, maroon or pink colour, either in the centre of the colony or throughout, are counted as presumptive faecal streptococci.

5.2 Confirmation

Confirmation on a more selective medium may be carried out if necessary.

The confirmation medium, bile-aesculin-azide agar, is incubated at 44 °C for 48 h. Faecal streptococci grow on this medium and hydrolyse aesculin; the end-product, 6,7-dihydroxycoumarin, combines with iron(III) ions to give a tan-coloured to black compound which diffuses into the medium.

Additionally a catalase test is performed on suspect colonies on a confirmatory medium.

Colonies which give a positive aesculin reaction and are catalase negative may be regarded as faecal streptococci.

6 Culture media and reagent

WARNING — All selective media described in this part of ISO 7899 contain sodium azide. As this substance is highly toxic and mutagenic, precautions should be taken to avoid contact with it, especially by the inhalation of fine dust during the preparation of commercially available dehydrated complete media. Azide-containing media should not be mixed with strong inorganic acids, as toxic hydrogen azide (HN_3) may be produced. Solutions containing azide can also form explosive compounds when in contact with metal pipework, for example from sinks.

6.1 Basic materials

For uniformity of results, in the preparation of media, use either constituents of uniform quality and chemicals of recognized analytical grade, or a dehydrated complete medium. Sodium azide deteriorates with time so that dehydrated media have a limited shelf-life. Use only distilled water or water of equivalent purity.

6.2 Culture media.

6.2.1 KF-streptococcus agar (Kenner)

6.2.1.1 Basal medium

proteose peptone	10,0 g
yeast extract	10,0 g
sodium chloride (NaCl)	5,0 g
sodium glycerophosphate	10,0 g
maltose	20,0 g
lactose	1,0 g
sodium azide (NaN_3)	0,4 g
bromocresol purple (ethanolic solution 15 g/l)	1 ml
agar	12 to 20 g [1]
water	up to 1 000 ml

Dissolve the ingredients in the water by heating in a boiling water-bath.

After dissolution is complete, heat for an additional 5 min.

Allow to cool to 50 to 60 °C.

6.2.1.2 TTC solution

2,3,5-triphenyltetrazolium chloride	1 g
water	100 ml

Dissolve the dye in the water by stirring.

Sterilize by filtration (0,22 µm).

The solution should be protected against the action of light.

6.2.1.3 Complete medium

basal medium (6.2.1.1)	1 000 ml
TTC solution (6.2.1.2)	10 ml

Add the TTC solution to the basal medium cooled to 50 to 60 °C. TTC is thermolabile, so that overheating must be avoided.

Adjust the pH if necessary to 7,2 with a sterile solution of sodium carbonate (100 g/l).

Pour the medium into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface.

Poured plates may be stored in the dark for up to 30 days at 4 ± 2 °C.

6.2.2 m-enterococcus agar (Slanetz and Bartley)

6.2.2.1 Basal medium

tryptose	20,0 g
yeast extract	5,0 g
glucose	2,0 g
dipotassium hydrogenorthophosphate (K_2HPO_4)	4,0 g
sodium azide (NaN_3)	0,4 g
agar	15,0 g
water	up to 1 000 ml

Dissolve the ingredients in the water by heating in a boiling water-bath.

After dissolution is complete, heat for an additional 5 min.

Cool to 50 to 60 °C.

6.2.2.2 TTC solution

See 6.2.1.2.

6.2.2.3 Complete medium

basal medium (6.2.2.1)	1 000 ml
TTC solution (6.2.2.2)	10 ml

Add the TTC solution to the basal medium cooled to 50 to 60 °C.

Adjust the pH if necessary to 7,2 with a solution of sodium carbonate (100 g/l).

1) According to the manufacturer's instructions.

Pour 20 ml into Petri dishes of 9 cm diameter (or an equivalent amount in a dish of another size) and allow to set on a cool, horizontal surface.

Poured plates may be stored in the dark for up to 30 days at 4 ± 2 °C.

6.2.3 Bile-aesculin-azide agar

tryptone	17,0 g
peptone	3,0 g
yeast extract	5,0 g
ox-bile, dehydrated	10,0 g
sodium chloride (NaCl)	5,0 g
aesculin	1,0 g
ammonium iron(III) citrate	0,5 g
sodium azide (NaN_3)	0,15 g
agar	12 to 20 g[1)]
water	up to 1 000 ml

Dissolve the ingredients in the water by boiling.

Adjust the pH so that after sterilization it will be 7,1 ± 0,1 at 25 °C.

Distribute in volumes of 250 ml in screw-capped bottles of 500 ml capacity.

Sterilize for 15 min at 121 ± 1 °C.

Cool to 50 to 60 °C and pour into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface.

6.3 **Hydrogen peroxide,** solution, 30 g/l.

7 Apparatus

Usual microbiological laboratory equipment and

7.1 Membrane filtration apparatus.

7.2 Sterile membrane filters, with a nominal pore size of 0,45 µm.

The quality of membrane filters may vary from brand to brand or even from batch to batch. It is therefore advisable to check the quality on a regular basis, according to ISO 7704.

7.3 Incubator, capable of being maintained at 35 ± 1 °C or 37 ± 1 °C.

7.4 Incubator, capable of being maintained at 44 ± 0,5 °C.

7.5 Autoclave, capable of being maintained at 121 ± 1 °C.

1) According to the manufacturer's instructions.

8 Sampling

See ISO 5667/1, ISO 5667/2 and ISO 5667/3.

9 Procedure

9.1 Treatment of samples

General procedures, such as the treatment of samples and preparation of dilutions, will form the subject of a future International Standard.

9.2 Filtration and incubation

A general description of the membrane filtration technique will form the subject of a future International Standard.

Filter a suitable volume of water.

Place the membrane filter on either KF-streptococcus agar (6.2.1) or m-enterococcus agar (6.2.2).

Incubate the plates at 35 ± 1 °C or 37 ± 1 °C for 44 ± 4 h.

9.3 Enumeration

After incubation, count all raised colonies which show a red, maroon or pink colour, either in the centre or throughout the colony. Consider these colonies as presumptive faecal streptococci.

NOTE — Occasionally bacteria other than group D streptococci may produce this type of colony. Elevation of the incubation temperature to 44 ± 0,5 °C, after an initial incubation for 5 ± 1 h at 37 ± 1 °C, may prevent the growth of these organisms.

9.4 Confirmation

Subculture a representative sample of typical colonies on a plate of bile-aesculin-azide agar (6.2.3).

Incubate at 44 ± 0,5 °C for 48 h.

Regard all plates showing a tan to black colour in the colonies and/or in the surrounding medium as giving a positive reaction.

9.5 Catalase test

Place a drop of hydrogen peroxide solution (6.3) on colonies on bile-aesculin-azide agar (6.2.3). Evolution of bubbles of oxygen indicates catalase-positive organisms. Only catalase-negative colonies should be considered as faecal streptococci.

NOTE — To eliminate errors due to false negative catalase reactions which may occur on the bile-aesculin-azide agar, the test may be repeated on a subculture on a non-selective medium.

10 Expression of results

A general description of expression of results and calculation of the number of bacteria in the sample, will form the subject of a future International Standard.

11 Test report

The test report shall include the following information:

a) a reference to this part of ISO 7899;

b) all details necessary for complete identification of the sample;

c) the selective medium, the incubation temperature and any confirmatory tests used;

d) the results as indicated in clause 10 as the number of presumptive faecal streptococci per volume of sample;

e) the number of colonies tested as well as the number confirmed as faecal streptococci.