TC 147

International Standard 7899/1

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Water quality — Detection and enumeration of faecal streptococci — Part 1: Method by enrichment in a liquid medium

Qualité de l'eau — Recherche et dénombrement des streptocoques fécaux — Partie 1: Méthode par enrichissement en milieu liquide

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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.

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Water quality — Detection and enumeration of faecal streptococci — Part 1: Method by enrichment in a liquid medium

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 reference method for the detection and enumeration of faecal streptococci in water by enrichment in a liquid medium.

2 Field of application

The method can be applied to all types of water, including turbid water.

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.

4 Definition

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, and that give a negative reaction in the catalase test.

5 Principle and reactions

The detection of faecal streptococci in a specified volume of sample requires the following two steps.

5.1 Enrichment culture

Incubation of the sample in the selective liquid medium azide glucose broth for 44 ± 4 h at 35 or 37 °C. Faecal streptococci grow in this medium and ferment glucose with the formation of acid, which causes a change in the colour of the pH indicator from purple to yellow.

5.2 Confirmation

All enrichment tubes showing positive reactions after 24 or 48 h are subcultured on a confirmatory medium to eliminate false positive reactions such as those by other Gram-positive cocci or rods. The confirmation medium, bile-aesculin-azide agar, is then 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 the 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 **Azide glucose broth** (single strength)

beef extract	4,5 g
tryptone	15,0 g
glucose	7,5 g
sodium chloride (NaCl)	7,5 g
sodium azide (NaN_3)	0,2 g
bromocresol purple (ethanolic solution 15 g/l)	1 ml
water	up to 1 000 ml

Dissolve the ingredients in the water by boiling.

Adjust the pH so that after sterilization it will be 7,2 ± 0,1 at 25 °C.

Distribute in tubes in 10 ml volumes.

Sterilize the medium for 15 min at 121 ± 1 °C.

NOTE — For the examination of samples of water of more than 1 ml, double strength broth should be prepared in volumes equal to those of the sample to be examined.

6.2.2 **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 7,2 ± 0,1 at 25 °C.

Distribute in suitable containers.

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

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

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

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 Enrichment

Add 1 ml of sample (or diluted sample) to 10 ml of azide glucose broth (6.2.1) and mix thoroughly.

Volumes greater than 1 ml should be added to the same volume of double strength broth.

1) According to the manufacturer's instructions.

Incubate at 35 ± 1 °C or 37 ± 1 °C for 22 ± 2 h.

Consider all tubes showing a yellow colour (throughout the whole tube or in the lower part of the tube only) as giving a positive reaction. Reincubate negative tubes for an additional 22 ± 2 h.

After this incubation even a faint colour change to reddish purple should be considered indicative of acid production. In order to improve the interpretation, the colour of the inoculated tube should be compared with the colour of an uninoculated tube.

For quantitative results the most probable number (MPN) method should be used.

9.3 Confirmation

Confirm each enrichment culture showing acid production as follows.

Streak a loopful of the resuspended enrichment broth on a plate of bile-aesculin-azide agar (6.2.2).

Incubate at 44 ± 0,5 °C for 44 ± 4 h.

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

9.4 Catalase test

Place a drop of hydrogen peroxide solution (6.3) on colonies on bile-aesculin-azide agar. 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 most probable numbers 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 method of confirmation used;

d) the results as indicated in clause 10 as the most probable number of faecal streptococci per volume of sample.