SLOVENSKI STANDARD

SIST ISO 9308-2:1998

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Kakovost vode - Ugotavljanje prisotnosti in ģtevila koliformnih organizmov, termotolerantnih koliformnih organizmov in verjetne Escherichia coli - 2. del: Metoda z veļ epruvetami (najbolj verjetno ģtevilo)

Water quality -- Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli -- Part 2: Multiple tube (most probable number) method

Qualité de l'eau -- Recherche et dénombrement des organismes coliformes, des organismes coliformes thermotolérants et des Escherichia coli présumés -- Partie 2: Méthode du nombre le plus probable

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Water quality — Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* —

Part 2:

Multiple tube (most probable number) method

Qualité de l'eau — Recherche et dénombrement des organismes coliformes, des organismes coliformes thermotolérants et des Escherichia coli *présumés —*

Partie 2: Méthode du nombre le plus probable

Reference number
ISO 9308-2:1990(E)

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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 9308-2 was prepared by Technical Committee ISO/TC 147, *Water quality*.

ISO 9308 consists of the following parts, under the general title *Water quality — Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli:*

— *Part 1: Membrane filtration method*

— *Part 2: Multiple tube (most probable number) method*

Annexes A and B of this part of ISO 9308 are for information only.

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Introduction

The presence and extent of faecal pollution is an important factor in assessing the quality of a body of water. Examination of water samples for the presence of members of the coliform group of organisms[1], which normally inhabit the bowel of man and other warm-blooded animals, provides an indication of such pollution. As the ability of some members of the coliform group of organism to survive in water is limited, their numbers can also be used to estimate the degree of recent faecal pollution.

1) See annex A for further microbiological information relevant to water examination for the coliform group of organisms.

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Water quality — Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* —

Part 2:
Multiple tube (most probable number) method

1 Scope

This part of ISO 9308 specifies a method for the detection and enumeration in water of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* (presumptive *E. coli*) by culture in a liquid medium in multiple tubes and calculation of their most probable numbers in the sample.

This method can be applied to all types of water, including those containing an appreciable amount of suspended matter.

The choice of tests used in the detection and confirmation of the coliform group of organisms, including *E. coli*, can be regarded as part of a continuous sequence. The extent of confirmation with a particular sample depends partly on the nature of the water and partly on the reasons for the examination. In practice, the detection in water of presumptive *E. coli* as defined in 3.3 of this part of ISO 9308, usually provides an indication of recent faecal pollution.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 9308. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 9308 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2:1982, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-3:1985, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.*

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 8199:1988, *Water quality — General guide to the enumeration of micro-organisms by culture.*

3 Definitions

For the purposes of this part of ISO 9308, the following definitions apply.

3.1 coliform organisms: Organisms capable of aerobic growth at either 35 °C ± 0,5 °C or 37 °C ± 0,5 °C in a liquid lactose culture medium with the production of acid and gas within 48 h.

3.2 thermotolerant coliform organisms: Coliform organisms as described in 3.1 which have the same fermentative properties within 24 h, at either 44 °C ± 0,25 °C or 44,5 °C ± 0,25 °C.

3.3 presumptive *Escherichia coli* (presumptive *E. coli*): Thermotolerant coliform organisms as described in 3.2 which also produce indole from tryptophan within 24 h, at either 44 °C ± 0,25 °C or 44,5 °C ± 0,25 °C.

4 Principle

Inoculation of test portions of the sample, diluted or undiluted, into a series of tubes of a selective liquid culture medium containing lactose.

Examination of the tubes after 24 h and 48 h incubation at either 35 °C or 37 °C; subculture from each tube showing turbidity with gas production into a more selective confirmatory medium and, when presumptive *E. coli* is sought, to a medium in which the formation of indole can be demonstrated.

Incubation of these confirmatory media for up to 48 h at either 35 °C or 37 °C for the detection of coliform organisms, and at 44 °C for up to 24 h for thermotolerant coliform organisms and presumptive *E. coli*.

By means of statistical tables, calculation of the most probable numbers (MPN) of coliform organisms, thermotolerant coliform organisms and presumptive *E. coli* likely to be present in 100 ml of the sample, from the number of tubes giving positive confirmatory results.

5 Diluent, culture media and reagents

5.1 Basic materials

Use ingredients of uniform quality and chemicals of analytical grade for the preparation of culture media and reagents and follow the instructions given in annex B. For information on storage see ISO 8199. Alternatively, use dehydrated complete media and follow strictly the manufacturer's instructions.

For the preparation of media, use glass-distilled water or de-ionized water free from substances which might inhibit bacterial growth under the conditions of the test, and in accordance with ISO 3696.

5.2 Diluent

For making sample dilutions, use one of the diluents recommended in annex B . Prepare the diluent according to the instructions given in annex B.

5.3 Isolation media

Use one of the following culture media. Instructions for preparing the media are given in annex A.

5.3.1 Lactose broth

5.3.2 MacConkey broth

5.3.3 Improved formate lactose glutamate medium[2)]

5.3.4 Lauryl tryptose (lactose) broth

5.4 Confirmatory media

Use one or more of the following.

5.4.1 Media for gas production

5.4.1.1 Brilliant-green lactose (bile) broth

5.4.1.2 EC medium

5.4.2 Medium for indole production

Tryptone water.

5.4.3 Single-tube medium for both gas and indole production

Lauryl tryptose mannitol broth with tryptophan.

5.5 Reagents

5.5.1 Kovacs' reagent for indole

5.5.2 Oxidase reagent for the oxidase test

6 Apparatus

Usual microbiological laboratory equipment, including

6.1 Hot-air oven for dry-heat sterilization and an autoclave.

Apart from apparatus supplied sterile, glassware and other equipment shall be sterilized according to the instructions given in ISO 8199.

6.2 Incubator or water bath, thermostatically controlled at either 35 °C ± 0,5 °C or 37 °C ± 0,5 °C.

6.3 Incubator or water bath, thermostatically controlled at either 44 °C ± 0,25 °C or 44,5 °C ± 0,25 °C.

6.4 pH meter.

2) Available commercially in dehydrated form as "Minerals modified glutamate medium". This information is given for the convenience of users of this part of ISO 9308 and does not constitue an endorsement by ISO of this product.

7 Sampling

Take the samples and deliver them to the laboratory in accordance with ISO 8199, ISO 5667-1, ISO 5667-2 and ISO 5667-3.

8 Procedure

8.1 Preparation of the sample and inoculation of media

For preparation of the sample, making dilutions and inoculation of isolation medium with test portions, follow the instructions given in ISO 8199. For test portions of volume greater than 5 ml, use tubes containing "double strength" isolation medium.

8.2 Incubation of tubes

Incubate the inoculated tubes for 48 h at either 35 °C ± 0,5 °C or 37 °C ± 0,5 °C.

8.3 Examination of tubes

Examine the tube-cultures after incubation for 18 h to 24 h and regard as positive reactions those which show turbidity due to bacterial growth and gas formation in the inner inverted (Durham) tubes, together with acid production if the isolation medium contains a pH indicator. Reincubate those tubes which do not show any or all of these changes and examine them again for positive reactions after 48 h.

8.4 Confirmatory tests

It is important to note that positive reactions in tubes of isolation medium are only presumptive coliform results. It is therefore important to carry out confirmatory tests, preferably on pure subcultures.

8.4.1 Subculture, incubation and examination

Subculture from each tube of isolation medium giving a positive result into one or more tubes of the confirmatory media (5.4) for gas and indole production.

NOTE 1 If the least inhibitory medium (lactose broth) is used for isolation, subculture to either of the two more selective confirmatory media [brilliant-green lactose (bile) broth or EC broth] for confirmation is recommended.

8.4.1.1 Coliform organisms

To confirm the presence of coliform organisms, incubate one tube of brilliant-green lactose (bile) broth (5.4.1) at either 35 °C or 37 °C, and examine for gas production within 48 h.

8.4.1.2 Thermotolerant coliform organisms and presumptive *E. coli*

To confirm the presence of thermotolerant coliform organisms, incubate another tube of EC medium (5.4.1) at 44 °C for 24 h, and examine for gas production.

To confirm the presence of presumptive *E. coli*, incubate a tube of tryptone water (5.4.2) for indole formation at 44 °C for 24 h. Then add 0,2 ml to 0,3 ml of Kovacs' reagent (5.5.1) to the tube of tryptone water: development of a red colour after gentle agitation denotes the presence of indole.

NOTES

2 The use of lauryl tryptose mannitol broth with tryptophan allows both gas and indole production by presumptive *E. coli* to be demonstrated in a single tube.

3 The detection of presumptive *E. coli* is regarded as satisfactory evidence of faecal pollution. However, further tests for the confirmation of *E. coli* may be carried out if considered necessary (see 8.5).

4 When subculturing from colonies on the membrane to tubes of confirmatory media, it is preferable to subculture also to a plate of nutrient agar medium for the oxidase test.

8.5 Oxidase test

Some bacteria found in water may conform to the definition of coliform organisms in most respects, but are able to produce gas from lactose only at temperatures below 37 °C. They therefore give negative results in the standard confirmatory tests for coliform organisms and their presence in water is not usually regarded as significant. *Aeromonas* species, which occur naturally in water, interfere with the determination only at a temperature of 37 °C and below, confirmation with the oxidase test is needed only when determining coliforms.

8.5.1 Carry out the oxidase test with pure subcultures of lactose-fermenting organisms, grown on nutrient agar medium, as follows:

- place 2 or 3 drops of freshly prepared oxidase reagent (5.5.2) on a filter paper in a Petri dish;
- with a glass rod, swab stick or platinum (not nichrome) wire loop, smear some of the growth on the prepared filter paper (see note 4);
- regard the appearance of a deep blue-purple colour within 10 s as a positive reaction.

NOTE 5 On each occasion that the oxidase reagent is used, control tests should be conducted with cultures of an organism known to give a positive reaction (*Pseudomonas aeruginosa*) and a negative reaction (*E. coli*).