



DRAFT INTERNATIONAL STANDARD ISO/DIS 9308-1

ISO/TC 147/SC 4

Secretariat: DIN

Voting begins on
2012-11-22

Voting terminates on
2013-04-22

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

Water quality — Enumeration of *Escherichia coli* and coliform bacteria —

Part 1:

Membrane filtration method for waters with low bacterial background flora

Qualité de l'eau — Dénombrement des Escherichia coli et des bactéries coliformes —

Partie 1: Méthode par filtration sur membrane dans des eaux contenant peu de flore bactérienne de fond

[Revision of first edition (ISO 9308-1:2000) and ISO 9308-1:2000/Cor 1:2007]

ICS 07.100.20

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO-lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five-month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

THIS DOCUMENT IS A DRAFT CIRCULATED FOR COMMENT AND APPROVAL. IT IS THEREFORE SUBJECT TO CHANGE AND MAY NOT BE REFERRED TO AS AN INTERNATIONAL STANDARD UNTIL PUBLISHED AS SUCH.

IN ADDITION TO THEIR EVALUATION AS BEING ACCEPTABLE FOR INDUSTRIAL, TECHNOLOGICAL, COMMERCIAL AND USER PURPOSES, DRAFT INTERNATIONAL STANDARDS MAY ON OCCASION HAVE TO BE CONSIDERED IN THE LIGHT OF THEIR POTENTIAL TO BECOME STANDARDS TO WHICH REFERENCE MAY BE MADE IN NATIONAL REGULATIONS.

RECIPIENTS OF THIS DRAFT ARE INVITED TO SUBMIT, WITH THEIR COMMENTS, NOTIFICATION OF ANY RELEVANT PATENT RIGHTS OF WHICH THEY ARE AWARE AND TO PROVIDE SUPPORTING DOCUMENTATION.

iTeh STANDARD PREVIEW
(standards.iteh.ai)
Full standard:
<https://standards.iteh.ai/catalog/standards/sist/649c6728-9364-42e9-b093-7163d0abf5c0/iso-9308-1-2014>

Copyright notice

This ISO document is a Draft International Standard and is copyright-protected by ISO. Except as permitted under the applicable laws of the user's country, neither this ISO draft nor any extract from it may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, photocopying, recording or otherwise, without prior written permission being secured.

Requests for permission to reproduce should be addressed to either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Reproduction may be subject to royalty payments or a licensing agreement.

Violators may be prosecuted.

Contents

Page

Foreword	iv
Introduction.....	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle.....	2
5 Apparatus and glassware	2
6 Culture media and reagents	3
7 Sampling.....	3
8 Procedure	3
9 Expression of results	4
10 Test report.....	4
11 Quality assurance.....	4
Annex A (informative) Further microbiological information on coliform bacteria	6
Annex B (normative) Culture media and reagents	7
Bibliography.....	10

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 9308-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This third edition cancels and replaces the second edition (ISO 9308-1:2000), which has been technically revised, and the Technical Corrigendum ISO 9308-1:2000/Cor.1:2007.

ISO 9308 consists of the following parts, under the general title *Water quality — Enumeration of Escherichia coli and coliform bacteria*:

- *Part 1: Membrane filtration method for waters with low bacterial background flora*
- *Part 2: Most probable number method*
- *Part 3: Miniaturized method (Most Probable Number) by inoculation in liquid medium*

Introduction

The presence and extent of faecal pollution is an important factor in assessing the quality of a body of water and the risk to human health from infection. Examination of water samples for the presence of *Escherichia coli* (*E. coli*), which normally inhabits the bowel of man and other warm-blooded animals, provides an indication of such pollution. Examination for coliform bacteria can be more difficult to interpret because some coliform bacteria live in soil and surface fresh water and are not always intestinal. Therefore, the presence of coliform bacteria, although not a proof of faecal contamination, may indicate failure in treatment or distribution.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Full standard:
<https://standards.iteh.ai/catalog/standards/sist/649c6728-9364-42e9-b093-7163d0abf5c0/iso-9308-1-2014>

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Full standard:
<https://standards.iteh.ai/catalog/standards/sist/649c6728-9364-42e9-b093-7163d0abf5c0/iso-9308-1-2014>

Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora

WARNING – Persons using this part of ISO 9308 should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT – It is absolutely essential that tests conducted in accordance with this part of ISO 9308 be carried out by suitably qualified staff.

1 Scope

This part of ISO 9308 specifies a method for the enumeration of *E. coli* and coliform bacteria. The method is based on membrane filtration, subsequent culture on a chromogenic agar medium and calculation of the number of target organisms in the sample. Due to the low selectivity of the differential agar medium, background growth can interfere with the reliable enumeration of coliform bacteria and *E. coli*, for example in surface waters or shallow well waters. This method is not suitable for these types of water.

This part of ISO 9308 is especially suitable for waters with low bacterial numbers that will cause less than 80 total colonies on chromogenic coliform agar. These may be drinking water, disinfected pool water or finished water from treatment plants.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

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

ISO 7704, *Water quality — Validation of membrane filters for microbiological analysis*

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

ISO 11133, *Microbiology of food, animal feeding stuffs, food production environment and water — Preparation, production, storage and performance testing of culture media*

ISO 19458, *Water quality — Sampling for microbiological analysis*

3 Terms and definitions

For the purpose of this document, the definitions given in ISO/IEC Guide 2 and the following apply.

3.1

coliform bacteria

members of the Enterobacteriaceae that express β -galactosidase

3.2

Escherichia coli

member of the Enterobacteriaceae that expresses β -galactosidase and β -glucuronidase

4 Principle

Filtration of a test portion of the sample through a membrane composed of cellulose esters or equivalent, which retains the organisms; placing of the membrane on a chromogenic agar plate.

Incubation of the membrane for (21 ± 3) h at (36 ± 2) °C.

Counting of β -galactosidase positive colonies (salmon to red) as presumptive coliforms that are not *E. coli*. To avoid false-positive results, caused by oxidase positive bacteria like *Aeromonas spp*, the presumptive colonies shall be confirmed by a negative oxidase reaction.

Counting of β -galactosidase and β -glucuronidase positive colonies (dark-blue to violet) as *E. coli*.

Total coliforms are the sum of oxidase negative colonies with salmon to red colour and all dark-blue to violet colonies.

5 Apparatus and glassware

Usual microbiological laboratory equipment, and in particular:

5.1 Apparatus for sterilization by steam (autoclave)

Apparatus and glassware not supplied sterile shall be sterilized according to the instructions given in ISO 8199.

5.2 **Incubator**, thermostatically controlled at (36 ± 2) °C.

5.3 **pH meter**, with an accuracy of $\pm 0,1$ at 20 °C to 25 °C.

5.4 **Equipment for membrane filtration**, as specified in ISO 8199.

5.5 **Membrane filters**, composed of cellulose esters, usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 μ m and, preferentially, with grids.

The filters shall be free from growth-inhibiting or growth-promoting properties and the printing ink used for the grid shall not affect the growth of bacteria. If not obtained sterile, they shall be sterilized according to the manufacturer's instructions. Every batch of membranes shall be tested for its suitability for the test according to ISO 7704 especially since the use of different brands of filters may result in different recovery and colour development.

5.6 **Forceps with rounded tips for handling membranes**

6 Culture media and reagents

For the preparation of culture media and reagents, use ingredients of uniform quality and chemicals of analytical grade (see note); follow the instructions given in Annex B. Alternatively, use commercially available media and reagents which comply with the compositions given in Annex B and follow strictly the manufacturer's instructions.

NOTE The use of chemicals of other grades is possible providing they are shown to be of equal performance in the test.

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

Unless specified otherwise, the prepared media are stable for at least one month if stored in the dark at $(5 \pm 3) ^\circ\text{C}$ and protected against evaporation.

7 Sampling

Take the samples and deliver them to the laboratory in accordance with ISO 19458.

8 Procedure

8.1 Preparation of the sample

For preparation of the sample, filtration and inoculation on isolation media, follow the instructions given in ISO 8199. Samples have to be transported and stored at $(5 \pm 3) ^\circ\text{C}$ in accordance with ISO 19458. Under exceptional circumstances, the samples may be kept at $(5 \pm 3) ^\circ\text{C}$ for up to 24 h prior to examination.

8.2 Filtration

Filter 100 ml (or other volumes, e.g. 250 ml for bottled water) of the sample to be studied using a membrane filter (5.5).

8.3 Incubation and differentiation

After filtration (8.2) place the membrane on the Chromogenic Coliform Agar (B.1) ensuring that no air is trapped underneath and incubate at $(36 \pm 2) ^\circ\text{C}$ for (21 ± 3) h.

Examine the membranes and count all colonies giving a positive β -galactosidase reaction (salmon to red) as presumptive coliform bacteria that are not *E. coli*.

Count all colonies giving a positive β -galactosidase and β -glucuronidase reaction (dark-blue to violet) as *E. coli*.

To confirm the presumptive coliform bacteria that are not *E. coli*, an oxidase test has to be performed. Test preferentially all, or at least 10 colonies from a subarea of the salmon to red colonies of the filter. For this confirmation step, appropriate commercialised oxidase-tests, like the Bactident[®] 1) Oxidase test can be used.

1) Bactident[®] is a registered trademark. Tradenames of products are examples of suitable products available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.