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Water quality — General requirements and guidance for microbiological examinations by culture

Qualité de l'eau — Exigences et lignes directrices générales pour les examens microbiologiques sur milieu de culture

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

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document 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 8199:2005), which has been technically revised. The main changes compared with the previous edition are as follows.

— Clauses have been added for terms and definitions, detection (qualitative) methods, performance characteristics and analytical quality control (AQC).

— The clauses referencing culture media and diluent preparation and QC have been revised to align with ISO 11133 and have been included in a new Annex D.

— The subclause on general guidance for the calculation of results for solid media techniques has been updated to reflect the changes in ISO 7218:2007/Amd.1:2013[9] on which the relevant clauses and subclauses in the second edition were based. Modifications have been made, however, to take account of water microbiology techniques (e.g. membrane filtration) and to allow for dilutions other than ten-fold dilutions.

— Annex B has been added to give guidance on confidence intervals when calculating special cases, relating to the update of the subclause on general guidance for the calculation of results for solid media techniques.

— Annex C has been added to describe calculations when using duplicate dishes per dilution, relating to the update of the subclause on general guidance for the calculation of results for solid media techniques.

— The subclause relating to enumeration using liquid media had been expanded and includes additional guidance on the use of MPN calculators. The former Annex B containing MPN tables has been removed.

— The title of this document has been amended to reflect these changes.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Techniques for the detection and enumeration of microorganisms based on their ability to grow on or in specified culture media are an important and widely used means of assessing the microbiological quality of water. The purpose of this document is to gather in a single document the information common to the various techniques. This reduces repetition of technical details in individual standards and facilitates choice of the technique most suitable for a particular situation. Other guidance has been included on general topics of relevance to these techniques, such as analytical quality control, method performance characteristics and uncertainty of test results.

Water quality — General requirements and guidance for microbiological examinations by culture

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted according to this document be carried out by suitably trained staff.

1 Scope

This document specifies requirements and gives guidance for performing the manipulations common to each culture technique for the microbiological examination of water, particularly the preparation of samples, culture media, and general apparatus and glassware, unless otherwise required in the specific standard. It also describes the various techniques available for detection and enumeration by culture and the criteria for determining which technique is appropriate.

This document is mainly intended for examinations for bacteria, yeasts and moulds, but some aspects are also applicable to bacteriophages, viruses and parasites. It excludes techniques not based on culturing microorganisms, such as polymerase chain reaction (PCR) methods.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 11133, *Microbiology of food, animal feed 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 purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
accuracy
closeness of agreement between a test result and the accepted reference value

[SOURCE: ISO 6107-8:1993, 1, modified — The note has been deleted.]

3.2
bias
difference between the expectation of the test results and an accepted reference value

[SOURCE: ISO 5725-1:1994, 3.8, modified — Note 1 to entry has been deleted.]

3.3
confirmed count
count (3.4) corrected for not confirmed *presumptive counts* (3.9) by further testing of the presumptive objects

3.4
count
<microbiology> observed number of objects such as colonies or cells determined by direct counting, or most probable number (MPN) estimation based on statistical calculation using the number of positive units or presumptive positive units in a dilution series of a *test sample* (3.16)

[SOURCE: ISO 6107-6:2004, 22, modified — "or presumptive positive units" has been added.]

3.5
detection level
minimum concentration of organisms that produce evidence of growth with a probability of p = 0,95 when inoculated into a specified culture medium and incubated under defined conditions

Note 1 to entry: The theoretical level that conforms to this definition is an average of three viable cells in an inoculum volume.

[SOURCE: ISO 13843:2017, 3.10]

3.6
intralaboratory reproducibility
intermediate precision

closeness of agreement between test results obtained with the same method on the same or similar test materials in the same laboratory with different operators using different equipment

3.7
limit of determination
lowest analyte concentration per analytical portion where the expected relative standard uncertainty equals a specified value

[SOURCE: ISO 13843:2017, 3.17]

3.8
precision
closeness of agreement between independent test results obtained under stipulated conditions

[SOURCE: ISO 5725-1:1994, 3.12, modified — Notes 1 to 3 to entry have been deleted.]

3.9
presumptive count
colony *count* (3.4) or most probable number (MPN) estimate based on the number of colonies or reaction vessels that have an outward appearance that is interpreted as typical of a target organism

[SOURCE: ISO 6107-6:2004, 62, modified — "fermentation tubes" has been replaced with "reaction vessels".]

3.10
relative standard deviation
u_{rel}
estimate of the standard deviation of a population from a sample of n results divided by the mean of that sample

[SOURCE: ISO 13843:2017, 3.30]

3.11
repeatability
measurement repeatability
measurement *precision* (3.8) under a set of *repeatability conditions* (3.12) of measurement

[SOURCE: ISO 13843:2017, 3.32]

3.12
repeatability conditions
condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

[SOURCE: ISO 13843:2017, 3.33]

3.13
reproducibility
measurement reproducibility
measurement *precision* (3.8) under *reproducibility conditions* (3.14) of measurement

Note 1 to entry: Relevant statistical terms are given in ISO 5725-1 and ISO 5725-2.

[SOURCE: ISO 13843:2017, 3.34]

3.14
reproducibility conditions
condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

[SOURCE: ISO 13843:2017, 3.35]

3.15
test portion
specified quantity of the sample that is taken for analysis

EXAMPLE 0,1 ml, 1 ml, 100 ml of sample.

3.16
test sample
undiluted, diluted or otherwise prepared *test portion* (3.15) of a sample to be tested, after completion of all preparation steps such as centrifugation, filtration, homogenization, pH adjustment and determination of ionic strength

[SOURCE: ISO 6107-6:2004, 92, modified — Note 1 to entry has been deleted.]

3.17
trueness
closeness of agreement between the average value obtained from a large series of test results and an accepted reference value

Note 1 to entry: The measure of trueness is usually expressed in terms of *bias* (3.2).

[SOURCE: ISO 6107-8:1993, 63]

3.18
uncertainty of counting
relative standard deviation (3.10) of results of repeated counting of the colonies or particles of the same plate(s) or field(s) under stipulated conditions (same person, different persons in one laboratory, or different laboratories)

[SOURCE: ISO 6107-6:2004, 103, modified — The domain has been deleted.]

4 Principle

The general principle of these techniques consists of inoculating a test portion of a water sample, or resultant test sample following membrane filtration or centrifugation, on or into a culture medium (solid or liquid). It is assumed that after incubation each target microorganism present multiplies, giving either a colony visible directly on or in the solid medium or changes in the observable properties of the liquid medium. The choice of a particular culture method depends not only on the nature and numbers of the microorganisms sought, but also on the nature of the water and the reasons for the examination.

5 General measurement requirements

5.1 Uniformity of temperatures

The following accepted ranges of temperatures and their ranges for incubation or storage are applied, where appropriate for the intended target organism and unless otherwise required in the specific standard.

Storage temperatures: (−70 ± 10) °C; (−20 ± 5) °C; (5 ± 3) °C

Incubation temperatures: (22 ± 2) °C; (36 ± 2) °C; (44 ± 0,5) °C

Media tempering temperature: 44 °C to 47 °C

The upper incubation temperature limits shall be followed strictly to ensure optimal growth. The lower temperature limits may be exceeded for short periods, e.g. due to opening the door of an incubator, but recovery to the operating temperature should be rapid.

5.2 Incubation times

The following accepted ranges of incubation times are applied, where appropriate for the intended target organism and unless otherwise required in the specific standard.

Incubation times: (21 ± 3) h; (44 ± 4) h; (68 ± 4) h

5.3 Volumes and masses

Measuring equipment shall be appropriate to the required accuracy and precision. The accepted range of any measured value is ±2 % where the stated value is critical to method performance and test results, and ±5 % where the stated value has been shown not to be critical. Examples of critical values having a direct effect on test results are inoculum and diluent volumes. For tolerances relating to the mass of ingredients used to prepare culture media, refer to ISO 11133.

NOTE Critical tolerances have been set at 2 % to minimize the uncertainty of test results.

6 Diluents and culture media

6.1 General

General requirements for preparation, production, sterilization, storage and performance of culture media are given in ISO 11133.

For preparation of microbiological culture media, unless otherwise stated, add the ingredients to the volume of water, rather than make the ingredients up to a certain volume.

Before use, check the quality of the culture media, diluents, membrane filters and reagents by following the procedures described in ISO 11133 and ISO 7704 or as given in the specific standard.

For information on storage of culture media, refer to ISO 11133.

6.2 Quality requirements of ingredients

Use constituents of uniform quality and analytical grade chemicals for the preparation of media. Other grades of chemicals may be used provided they can be shown to produce equivalent results. Alternatively, dehydrated complete media or diluents may be used. Follow the manufacturer's instructions strictly.

Refer to ISO 11133 and ISO 3696[2] for further information on the quality of ingredients and the quality of water that should be used for media preparation.

6.3 Diluents

The following diluents are commonly used in water microbiology. However, other appropriate diluents may be used and this list is not exhaustive:

— saline solution;

— peptone diluent;

— peptone saline solution [maximum recovery diluent (MRD)];

— quarter-strength Ringer's solution;

— phosphate buffer solution.

Follow the formulations and the preparation, storage and performance testing instructions given in Annex D for these diluents.

7 Sterilization and decontamination

7.1 Sterilization of apparatus and glassware

Sterilize apparatus and glassware not supplied sterile by one of the following methods:

a) in an oven, operating at (170 ± 10) °C for at least 1 h (excluding pre-heating time);

b) in an autoclave, operating at (121 ± 3) °C for at least 15 min.

Some heat labile items may require sterilization by other means (e.g. ultraviolet light or irradiation) but these are not carried out in the routine laboratory.

7.2 Sterilization of consumables

Sterile disposable equipment and materials may be used instead of re-usable items (glassware, Petri dishes, pipettes, bottles, tubes, loops, spreaders, etc.) if the specifications are similar.

If membrane filters are not obtained sterile, these are usually sterilized by moist heat according to process b) described in 7.1, or by following the manufacturer's instructions.

7.3 Decontamination of glassware and materials after use

Materials for decontamination and disposal should be placed in appropriate containers, e.g. autoclavable plastic bags. Autoclaving is the preferred method for all decontamination processes (at least 30 min at 121 °C). The autoclave should be loaded in a way that favours heat penetration into the load (e.g. without over packing). Take care to loosen caps/lids and open bags to prevent dangerous pressurization of the container, which could lead to possible breakage, e.g. explosion of glass bottles.

Modern autoclaves may not require caps to be loosened, but follow the manufacturer's instructions strictly to avoid dangerous pressurization of the containers.

Alternative methods other than autoclaving may be used.

Autoclave all equipment which has been in contact with microbiological cultures (solid or liquid culture media), including re-usable containers prior to being washed.

During examination, decontamination by immersion in freshly prepared disinfectant, prepared at the correct dilution, may be used for small-sized and corrosion resistant equipment (e.g. pipettes).

Pasteur pipettes may be difficult to clean and are usually discarded after a single use.

Most disinfectants have some toxic effects. Wear gloves and eye protection when handling disinfectant and follow the manufacturer's instructions.

7.4 Waste management

The correct disposal of contaminated materials does not directly affect the quality of sample analysis, but it is a matter of good laboratory management. A system for identification and separation of waste materials and their containers should be established for:

— non-contaminated waste (e.g. uncultured water samples) that can be disposed of using general waste streams;

— scalpels, needles, knives and broken glass;

— contaminated materials for autoclaving and recycling;

— contaminated materials for autoclaving and disposal, or for disposal only if the material is to be incinerated.

Materials contaminated with risk category 3 microorganisms and their containers shall be autoclaved before they are incinerated.

8 Samples and sample handling

8.1 Sampling

Take samples in accordance with ISO 19458. Collect disinfected water samples in sample bottles containing suitable and sufficient neutralizers.

8.2 Sample preparation

8.2.1 Waters and other aqueous matrices

Clean and dirty water should be separated and processed using separate equipment in separate areas to reduce cross-contamination risk where possible. Alternatively, process batches of clean waters before dirty water.

Before examination, mix the sample thoroughly by agitation to achieve uniform distribution of microorganisms and other particles. This can be achieved by inversion of the sample or by a to-and-fro motion. Depending on the nature of the water and the microbial content anticipated, make any necessary dilutions at this stage.

For plate counts, ten-fold dilutions are usually used. For membrane filtration (with a smaller surface area), smaller dilution steps are recommended. For many most probable number (MPN) techniques, dilutions are an inherent part of the procedure. Refer to 9.2.3 for guidance on dilutions in MPN techniques. See ISO 6887-1[7] for general guidance on the preparation of serial dilutions.

For ten-fold dilutions, aseptically measure nine volumes of the diluent and one volume of the water sample into sterile dilution bottles or tubes. Alternatively, volumes of diluent pre-sterilized in screw-capped bottles are used and volumes verified after autoclaving. One or more ten-fold dilutions are made by transferring one volume of water sample to nine volumes of diluent. Mix the solution thoroughly with a fresh pipette or by mechanical means and transfer one volume of this dilution to another nine volumes of diluent. Repeat these steps as many times as required. Prepare sufficient volumes of each dilution for all the tests to be carried out on each water sample.

For dilutions of other magnitudes, the volume of diluent to volume of sample is adjusted accordingly. For example, four-fold dilutions can be made as described above for ten-fold dilutions, only in this case one volume of water sample is mixed with three volumes of diluent. Another approach is to use a ten-fold dilution series, but filter both 10 ml and 30 ml volumes.

If the concentration of the target organism is expected to be high, hundred-fold dilution steps may be used by mixing one volume of water sample with 99 volumes of diluent, but such large intervals between measurements can adversely affect the reliability of the test results.

8.2.2 Swabs

8.2.2.1 General

Swabs are sometimes used for assessing water quality, e.g. investigation of biofilms, and may be tested by both quantitative and qualitative methods.

Different types of swabs are available for specific purposes, including stick and sponge swabs, with and without neutralizing agents for any disinfectants present. Where transport times before testing are likely to be extended, special transport swabs giving some protection to the organisms present are recommended. All types of swabs should be evaluated before use, as some have been found to be inhibitory to certain microorganisms. Further guidance on the use of swabs and swabbing techniques can be found in ISO 18593[18].

NOTE Dry stick swabs are not suitable for microbiological testing because they offer no protection against dehydration or disinfectant residues for any microorganisms present during transport and storage before testing.

8.2.2.2 Stick swab preparation

Stick swabs in neutralizing solution are mixed thoroughly, either manually or by vortexing, in the solution.

Transport swabs in agar are carefully removed from the packaging and immersed in a volume (usually 10 ml) of diluent. The stick is then broken or cut off to allow thorough mixing in the sealed bottle or