INTERNATIONAL STANDARD

ISO 6222

Second edition
1999-05-15

Water quality — Enumeration of culturable micro-organisms — Colony count by inoculation in a nutrient agar culture medium

Qualité de l'eau — Dénombrement des micro-organismes revivifiables — Comptage des colonies par ensemencement dans un milieu de culture nutritif gélosé

Reference number
ISO 6222:1999(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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6222 was prepared by the European Committee for Standardization (CEN) in collaboration with ISO Technical Committee TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Throughout the text of this document, read "...this European Standard..." to mean "...this International Standard...".

This second edition cancels and replaces the first edition (ISO 6222:1988), which has been technically revised.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

Printed in Switzerland

Foreword

The text of EN ISO 6222:1999 has been prepared by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 147 "Water quality".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 1999, and conflicting national standards shall be withdrawn at the latest by November 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

Waters of all kinds invariably contain a variety of micro-organisms derived from various sources, such as soil and vegetation, and estimation of the overall numbers provide useful information for the assessment and surveillance of water quality. Separate counts are usually made of the micro-organisms which are able to grow and form colonies on nutrient agar media at 36 °C and 22 °C.

Colony counts are useful for assessing the integrity of ground water sources and the efficiency of water treatment processes such as coagulation, filtration and disinfection and provide an indication of the cleanliness and integrity of the distribution system. They can also be used to assess the suitabillty of a supply for the preparation of food and drink, where the water supply should contain few micro-organisms to avoid contaminating the product with spoilage organisms.

The main value of colony counts lies in the detection of changes from those expected, based on frequent, long term monitoring. Any sudden increase in the count can be an early warning of serious pollution and calls for immediate investigation.

1 Scope

This European Standard specifies a method for the enumeration of culturable micro-organisms in water by counting the colonies formed in a nutrient agar culture medium after aerobic incubation at 36 °C and 22 °C.

The method is intended to measure the operational efficiency of the treatment process of public drinking water supplies and for general application to all types of water. It is particularly applicable to the examination of water intended for human consumption, including water in closed containers and to natural mineral waters.

2 Normative references

This European Standard incorporates provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed thereafter. For dated references, subsequent amendment to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the most recent edition of the publication referred to applies.

EN ISO 3696
Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)

EN ISO 5667-3
Water quality – Sampling – Part 3: Guidance on the preservation and handling of samples (ISO 5667-3:1994)

EN 25667-2
Water quality – Sampling – Part 2: Guidance on sampling techniques (ISO 5667-2:1991)

ISO 6887
Microbiology – General guidance for the preparation of dilutions for microbiological examinations

ISO 8199
Water quality – General guide to the enumeration of micro-organisms by culture

3 Definitions

For the purposes of this European Standard, the following definition applies:

culturable micro-organisms: All aerobic bacteria, yeasts and moulds capable of forming colonies in the medium specified under the test conditions described herein.

4 Principle

Inoculation by mixing with a specified culture medium in Petri dishes, measured volumes of the samples or dilutions of the sample. Incubation of one set of plates at 36 °C for 44 h, and another set at 22 °C for 68 h.

Calculation of the number of colony-forming units (c.f.u.) per millilitre (ml) of the sample from the number of colonies formed in the medium.

5 Apparatus and glassware

Usual microbiological laboratory equipment and, in particular:

5.1 Apparatus for sterilisation by steam (autoclave)

5.2 Incubator capable of maintaining a temperature of (36 ± 2) °C

5.3 Incubator capable of maintaining a temperature of (22 ± 2) °C

5.4 Glass or plastics Petri dishes with a diameter of 90 mm or 100 mm

5.5 Water bath or similar apparatus capable of maintaining a temperature of (45 ± 1) °C

5.6 Colony counting equipment with a method of illumination against a dark background

6 Sampling

Take the samples of water in accordance with the instructions for sampling, handling and preservation given in EN 25667-2 and EN ISO 5667-3.

Examine water supplied in closed containers, including natural mineral waters, within 12 h of bottling, keeping the temperature of storage at (5 ± 3) °C during this period.

7 Culture media and diluents

7.1 Basic materials

For the preparation of the medium, use ingredients of uniform quality and chemicals of analytical grade; alternatively use an equivalent dehydrated complete medium and follow the manufacturer's instructions.

For making media, use glass-distilled or deionised water prepared in accordance with EN ISO 3696 grade 3 and free from substances which might inhibit growth under the conditions of the test.

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

7.2 Diluent

For the dilutions, use the peptone diluent given in ISO 8199.

7.3 Yeast extract agar

Tryptone (Peptone from Casein, pancr.)	6,0 g
Dehydrated yeast extract	3,0 g
Agar, powdered or in pellets	10 g to 20 g (according to gel strength)
Water	1 000 ml

Add the ingredients, or the complete dehydrated medium, to the water and dissolve by heating. Adjust the pH if necessary so that after sterilization it will be 7,2 ± 0,2 at 25 °C.

Distribute volumes of 15 ml to 20 ml in tubes, bottles or other containers. For storage in larger volumes, use containers up to 500 ml capacity. Sterilise in the autoclave (5.1) at (121 ± 3)°C for (15 ± 1) minutes.

For use, melt the medium, allow to cool and maintain it at (45 ± 1) °C using the water bath (5.5). It is recommended to store the medium not longer than 4 h at 45 °C, after which time the medium shall be discarded.

8 Procedure

8.1 Preparation and inoculation

Carry out preparation of the sample, make dilutions and inoculate the culture media, in accordance with ISO 8199, EN ISO 5667-3 and ISO 6887.

Use the pour-plate method (ISO 8199). Place a volume of the test sample (or its dilution) not exceeding 2 ml in the Petri dish, add 15 ml to 20 ml of the molten medium (7.3) and mix carefully by gentle rotation; allow the medium to set. Time between addition of the test sample (or its dilution) and addition of the molten medium shall not exceed 15 min. Inoculate at least one plate for incubation at each temperature.

8.2 Incubation and examination

Invert the plates and incubate one set at (36 ± 2) °C for (44 ± 4) h; incubate the other set at (22 ± 2) °C for (68 ± 4) h. Examine the plates as soon as they are removed from the incubators; if this is not possible, store them at (5 ± 3) °C and examine them within 48 h. Reject any plate with confluent growth.

8.3 Counting of colonies

For each temperature of incubation, and following the procedures described in ISO 8199, count the colonies present in each plate and calculate the estimated number of colony forming units present in 1 ml of sample.

9 Expression of results

Express the results as the number of colony-forming units per millilitre (cfu/ml) of the sample for each temperature of incubation.

If there are no colonies in the plates inoculated with the test volumes of the undiluted sample, express the results as not detected in one millilitre. If there are more than 300 colonies on the plates inoculated with the highest dilutions used, express the results as > 300 or as approximate only.

10 Test report

The test report shall make reference to this European Standard and give all relevant information, including

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

b) the technique (pour plate) and medium used;

c) the time and temperature of incubation;

d) the results of the count expressed in accordance with clause 9;

e) any particular occurrence(s) observed during the course of the analysis and any other relevant facts concerning the procedure followed.

ICS 07.100.20

Price based on 4 pages