SLOVENSKI STANDARD
SIST ISO 6222:1997

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Kakovost vode - Štetje živih mikroorganizmov - Štetje kolonij z nasajanjem v hranljivi agar ali nanj

Water quality -- Enumeration of viable micro-organisms -- Colony count by inoculation in or on a nutrient agar culture medium

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

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INTERNATIONAL STANDARD

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

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

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

Reference number
ISO 6222 : 1988 (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 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.

International Standard ISO 6222 was prepared by Technical Committee ISO/TC 147, *Water quality*.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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

0 Introduction

Waters of all kinds invariably contain a variety of micro-organisms derived from various sources, and estimation of their overall numbers can provide useful information for the assessment and surveillance of water quality. Those micro-organisms able to survive in water can usually grow in laboratory culture media better at about 22 °C than at higher temperatures, the results often reflecting the environmental and seasonal conditions prevailing at the time. In contrast, micro-organisms which grow well at 37 °C generally survive in water only with difficulty and they are more likely to have come from other sources and have hygienic implications.

For these reasons, separate counts are usually made of the micro-organisms which are able to grow and form colonies on nutrient agar media under defined cultural conditions at each of these temperatures.

1 Scope and field of application

This International Standard specifies a method for the enumeration of viable micro-organisms in water by counting the colonies formed in or on a nutrient agar culture medium after aerobic incubation at 37 °C and/or at 22 °C.

The method is intended for general application to the microbiological examination of all types of water.

2 Reference

ISO 8199, *Water quality — General guide for microbiological examination — Enumeration of micro-organisms by culture.*

3 Definition

For the purpose of this International Standard, the following definition applies.

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

4 Principle

Inoculation by mixing with, or spreading on the surface of, a specified nutrient agar culture medium in Petri dishes, measured volumes of the sample or dilutions of the sample. Incubation of one set of plates at 37 °C for 24 h (or 48 h), and another set at 22 °C for 72 h.

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

5 Culture media and diluents

5.1 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 deionized water free from substances which might inhibit growth under the conditions of the test.

5.2 Diluent

For making the dilutions, use one of the diluents given in ISO 8199.

5.3 Yeast extract agar

Tryptone	6 g
Dehydrated yeast extract	3 g
Agar, powdered or in pellets	12 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 in tubes, bottles or other containers. For storage in larger volumes, use containers up to 500 ml capacity. Sterilize in the autoclave (6.1) at 121 °C for 15 min.

For use, melt the medium, allow to cool and maintain it at 45 ± 1 °C using the water bath (6.5).

6 Apparatus and glassware

Usual microbiological laboratory equipment, and in particular

6.1 Apparatus for sterilization by steam (autoclave).

6.2 Incubators capable of maintaining a temperature of 37 ± 1 °C.

6.3 Incubators capable of maintaining a temperature of 22 ± 1 °C.

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

6.5 Water bath, or similar apparatus capable of maintaining a temperature of 45 ± 1 °C.

6.6 Colony-counting equipment with a method of illumination against a dark background, a hand-lens (optional) and preferably a mechanical or electronic digital counter.

7 Sampling

Take the samples of water in accordance with the instructions given in ISO 8199.

8 Procedure

8.1 Preparation and inoculation

Carry out preparation of the sample, making dilutions and inoculation of culture media, in accordance with ISO 8199.

For pour plates, place the test volume in the Petri dish, add the molten medium (5.3) and mix carefully by gentle rotation; allow the medium to set. For spread plates, place the test volume on the dry surface of the agar medium (5.3) and distribute it over the surface with a sterile glass rod; allow the inoculum to be absorbed.

Inoculate at least two plates for each test volume at each temperature.

8.2 Incubation and examination

Invert the plates and incubate one set at 37 ± 1 °C for 24 ± 1 h or 48 ± 4 h; incubate the other set of plates at 22 ± 1 °C for 72 ± 4 h. Examine the plates as soon as they are removed from the incubators; if this is not possible, store them at 4 °C and examine them within 24 h. Reject any plate with confluent growth.

8.3 Counting of colonies

Count the colonies present in or on each plate, if necessary with magnification and the aid of a counting device (6.6).

Determine the average number of colonies from the pairs of plates from each dilution, each plate ideally containing between 25 and 300 colonies. For each temperature of incubation, calculate the estimated number of colony-forming units present in 1 ml of the sample.

Alternatively, if more than one pair of dilutions yields counts of between 25 and 300 colonies, then determine the weighted mean according to the formula given in 8.4 of ISO 8199. From these values estimate for each temperature of incubation the number of colony-forming units present in 1 ml of the sample.

9 Expression of results

Express the results as the number of colony-forming units per millilitre of the sample for each temperature of incubation.

If there are no colonies in or on the plates inoculated with test volumes of the undiluted sample, express the results as less than 1 colony-forming unit per millilitre. If there are more than 300 colonies on the plates inoculated with the highest dilutions used, express the results as approximate only.

10 Test report

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

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

b) the technique (pour plate or surface-spread 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.

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