INTERNATIONAL STANDARD

ISO 10718

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Cork stoppers — Enumeration of colony-forming units of yeasts, moulds and bacteria capable of growth in an alcoholic medium

Bouchons en liège — Dénombrement des unités formant colonie de levures, moisissures et bactéries capables de se développer dans un milieu alcoolique

Reference number
ISO 10718:1993(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 10718 was prepared by Technical Committee ISO/TC 87, *Cork*.

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Cork stoppers — Enumeration of colony-forming units of yeasts, moulds and bacteria capable of growth in an alcoholic medium

1 Scope

This International Standard specifies a method to enumerate the colony-forming units of yeasts, moulds and bacteria which can exist on cork stoppers and can grow in an alcoholic solution under certain conditions.

This International Standard applies to cork stoppers which were submitted to sanitizing procedures[1] and are contained in sealed packages.

2 Normative reference

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

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations.*

3 Principle

Direct counting of colonies of living micro-organisms (yeasts, moulds and bacteria) by incubation in malt extract broth, using a membrane filtration procedure.

1) The products used shall be admitted by the WHO.

2) This product is available commercially.

4 Reagents and incubation broth

4.1 WLN[2], with the following composition.

Bacto yeast extract	4,0 g
Bacto casitone	5,0 g
Bacto dextrose	50,0 g
Monopotassium phosphate	0,55 g
Potassium chloride	0,125 g
Calcium chloride	0,125 g
Magnesium sulfate	0,125 g
Ferric chloride	0,002 5 g
Manganese sulfate	0,002 5 g
Bacto-agar	20,0 g
Bacto-bromcresol green	0,022 g
Water to complete to	1 000 ml

NOTE 1 This product is very hygroscopic. The bottle must be kept tightly closed in a cool dry place.

4.2 Malt extract broth (rinsing solution)[2], pH 6,2 and with the following composition.

Bacto yeast extract	3,0 g
Malt extract, Difco	3,0 g
Bacto Peptone	5,0 g
Bacto dextrose	10,0 g

4.3 Tartaric acid.

4.4 Ethanol, spectrometric grade.

5 Apparatus

Usual microbiological laboratory apparatus (see ISO 7218) and, in particular, the following.

5.1 Vacuum filtration systems.

5.2 Sterile membranes, with 0,45 µm porosity.

6 Sampling

From each lot take, at random, 0,25 % of the sealed packages (minimum of three but not exceeding ten). From each package take eight cork stoppers and group them four by four.

7 Determination

7.1 Prepare the malt extract broth (4.2).

7.2 Adjust to pH 4,0 using tartaric acid (4.3).

7.3 Sterilize in an autoclave at 121 °C ± 1 °C at a pressure of 15 psi for 15 min.

7.4 Allow to cool and aseptically adjust the concentration to 8 % (*V/V*) with ethanol (4.4).

7.5 Aseptically dispense portions of 100 ml of this solution to 250 ml sterile flasks (two flasks per package plus one).

7.6 Prepare WLN (4.1) and dispense to sterile Petri dishes (two dishes per package plus one).

7.7 Aseptically take the cork stoppers from their package and place four in each flask using an aseptic procedure, ensuring that the stoppers are completely immersed.

7.8 Stir gently to displace air bubbles and keep each flask at 25 °C ± 1 °C for 24 h.

7.9 After that time, aseptically remove the cork stoppers and quickly filter (5.1) the contents of each flask through a membrane (5.2).

Incubate the membranes in WLN (7.6) for 5 d at 25 °C ± 1 °C.

7.10 Count the colonies at 24 h intervals and identify the type of microorganisms, if necessary.

8 Results

The mean number of colony-forming units of microorganisms per cork stopper is given by

$$\frac{N}{4}$$

where N is the total number of colonies counted in the Petri dish.

9 Blank test

Filter and incubate the contents of one additional flask, containing no cork stoppers, in WLN (7.6) under the same conditions. This allows a check to be made on the execution of the test.

10 Test report

The test report shall include the following information:

a) reference to this International Standard;

b) all details required to identify the sample;

c) the results obtained;

d) all details of procedure not specified in this International Standard or any optional operations;

e) any occurrences that have possibly affected the results.

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