INTERNATIONAL STANDARD

ISO 10718

Third edition
2015-10-15

Cork stoppers — Characterization of a low-in-germs stopper, through the enumeration of colony-forming units of yeasts, moulds and bacteria, capable of both being extracted and growing in alcoholic medium

Bouchons en liège — Caractérisation d'un bouchon pauvre en germes par dénombrement des unités formant colonie de levures, de moisissures et de bactéries, extraites en milieu alcoolique et capables de s'y développer

Reference number
ISO 10718:2015(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 87, *Cork*.

This third edition cancels and replaces the second edition (ISO 10718:2002), which has been technically revised.

Cork stoppers — Characterization of a low-in-germs stopper, through the enumeration of colony-forming units of yeasts, moulds and bacteria, capable of both being extracted and growing in 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 in an alcoholic solution, and which, under certain conditions, can be extracted during the 3 months following delivery.

This International Standard applies to all types of ready-to-use cork stoppers, submitted to a sanitation process and packaged in properly aseptic and hermetic conditions.

This International Standard specifies the limit values of the colony-forming units of yeasts, moulds and bacteria which can be found on cork stoppers submitted to the test procedures included in this standard.

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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 17727, *Cork — Cork stoppers for still wine — Sampling plan for the quality control of cork stoppers*

3 Low-in-germs stoppers

Cork stoppers submitted to test methods specified in this International Standard are designated as low-in-germs stoppers when the following results are obtained:

< 10 cfu bacteria per stopper (see 13.1)

< 10 cfu yeast and moulds per stopper (see 13.2)

4 Principle

Direct counting of colonies of living microorganisms (yeasts, moulds and bacteria) by incubation in a specific cultural medium after extraction with an alcoholic solution with added tartaric acid and followed by a membrane filtration procedure.

5 Reagents and cultural media

5.1 Physiological solution (0,85 % NaCl)[1] or **Ringer's solution** (1/4 X)[1] with the following composition:

1) This product is commercially available.

Sodium chloride	2,25 g/l
Potassium chloride	0,105 g/l
Calcium chloride $6H_20$	12 g/l
Sodium bicarbonate	0,05 g/l
Final pH (obtained from the mixture)	7,0 ± 0,2

5.2 WLD (for counting bacteria) with the following composition:

Yeast extract	4,0 g/l
Casein hydrolysate	5,0 g/l
Dextrose	50,0 g/l
Potassium dihydrogen phosphate	0,55 g/l
Potassium chloride	0,425 g/l
Calcium chloride	0,125 g/l
Magnesium sulfate	0,125 g/l
Manganese sulfate	0,002 5 g/l
Ferric chloride	0,002 5 g/l
Bromocresol green	0,022 g/l
Cycloheximide (actidione)	0,004 g/l
Final pH (obtained from the mixture)	5,5 ± 0,2

5.3 M-Green (for counting yeasts and moulds) with the following composition:

Yeasts extract	9,0 g/l
Dextrose (cerelose)	50,0 g/l
Peptone	10,0 g/l
Magnesium sulfate	2,10 g/l
Potassium phosphate	2,0 g/l
Diastase	0,05 g/l
Thiamine	0,05 g/l
Bromocresol green	0,026 g/l
Final pH (obtained from the mixture)	4,6 ± 0,2

5.4 Tartaric acid.

5.5 Ethanol, 96 %.

5.6 Wetting agent.

5.7 Triptone gel.

5.8 Diphenyl.

5.9 Demineralized water (or **water with a similar purity**).

Reagents and cultural media should be stored according to the manufacturer's instructions.

6 Apparatus

Usual microbiological laboratory apparatus and, in particular, the following.

6.1 Membrane filtration system.

One of the membrane filtration systems described at 6.1.1 or 6.1.2 may be used.

6.1.1 Sterile filtration system, ready-to-use, including a polypropylene funnel with at least a capacity of 100 ml, a sterile membrane (porosity 0,45 µm), a sterile dish and a vacuum pump with three-way cock to turn off the vacuum[2)].

6.1.2 Traditional filtration system, including a funnel with a minimum capacity of 100 ml (of stainless steel, glass or polycarbonate which can be sterilized in an autoclave or an oven), a sterile membrane (porosity 0,45 µm), a sterile Petri dish with blotting pad and a vacuum pump.

6.2 Incubator, capable of being controlled at 30 °C ± 2 °C.

6.3 Refrigerator, capable of being controlled at a temperature between 2 °C and 8 °C.

6.4 Orbital shaker, with a plate or a **wrist-action shaker** or a **rocking-motion shaker** that, depending on the model, can be set at a speed between 140 r/min and 160 r/min or 140 to 160 osc/min or 140 to 160 back-and-forth motions/min.

6.5 pH-meter with a temperature compensation, accurate to ± 0,1 at 25 °C.

6.6 Glass flasks, with screw caps and appropriate capacity to allow the four stoppers to be immersed in a 100 ml solution.

7 Sampling

Sampling shall be carried out aseptically according to ISO 17727.

Use sterile containers to preserve the sample at a temperature between 2 °C to 8 °C up to the time of testing.

8 Test condition

The preparation of the material and the test procedure shall be carried out aseptically and following the rules specified in ISO 7218.

2) This system is commercially available.

9 Extraction

9.1 Prepare the physiological solution or Ringer's solution (5.1). While stirring, add the wetting agent (5.6) to obtain a 10 g/l concentration and then add triptone gel (5.7) to obtain a 1 g/l concentration. Afterwards, adjust to a pH value between 3 and 3,5 using tartaric acid (5.4). Dispense about 90 ml of the solution to each flask (6.6) and sterilize.

9.2 After cooling, add to each flask, 10 ml of ethanol (5.5) aseptically.

9.3 Put four cork stoppers into each flask, checking that the cork stoppers are completely immersed. Shake the flasks for 1 h at a speed between 140 rpm to 160 rpm, and a temperature between 20 °C and 25 °C.

The number of flasks depends on the sampling plan that has been chosen. Half of the flasks are to be used for seeding on WLD and the remaining for seeding on M-Green.

For each cultural media, prepare an additional flask for the blank test.

10 Procedures

10.1 General

Follow procedure 10.2 when using a sterile filtration system and a sterile cultural media that is ready to use.

Follow procedure 10.3 when using a filtration system that has to be sterilized and a dehydrated cultural media.

10.2 Fast determination using a filtration system and a ready to use sterile culture media

10.2.1 Preparation

Prepare the filtration system (6.1.1).

10.2.2 Seeding on WLD

Place the complete funnel with the sterile membrane on the vacuum-pump filtration head. Aseptically filter the extraction solution prepared in accordance with Clause 9. At the end of the filtration, turn off the vacuum from the suction circuit to re-equilibrate the atmospheric pressure.

Just before the seeding, add diphenyl (5.8) dissolved in a 10 % ethanol solution to the WLD media (5.2) in order to obtain a 30 ppm diphenyl concentration. Add the WLD media contained in the ampoule suck it lightly and turn off the vacuum. Remove the filtration set and put the stopper on the base of the filtration set to avoid retro-infection. Take away the cylindrical part of the funnel. Lift the funnel cap and fit it on the set filter/Petri dish set.

Repeat this procedure for each flask.

10.2.3 Seeding on M-Green

Place the complete funnel with the sterile membrane on the vacuum-pump filtration head. Aseptically filter the extraction solution prepared in accordance with Clause 9. At the end of the filtration, turn off the vacuum from the suction circuit to re-equilibrate the atmospheric pressure.

Add the M-Green cultural media (5.3) contained in the ampoule, suck it lightly and turn off the vacuum. Remove the filtration set and put the stopper on the base of the filtration set to avoid retro-infection. Take away the cylindrical part of the funnel. Lift the funnel cap and fit it on the set filter/Petri dish set.

Repeat this procedure for each flask.

Dehydrated cultural media on the membrane shall be rehydrated using sterilized and demineralized water.

10.3 Determination using a filtration system to be sterilized and a dehydrated cultural media

10.3.1 Preparation of media

Prepare and sterilize the WLD media (5.2) and the M-Green media (5.3), following the manufacturer's instructions.

Add diphenyl (5.8), dissolved in a 10 % ethanolic solution, to the WLD media to obtain a 30 ppm concentration in diphenyl.

Prepare the Petri dishes.

10.3.2 Preparation of filtration system

Sterilize and prepare the filtration system (6.1.2).

10.3.3 Seeding on WLD

Aseptically filter the extraction solution prepared in accordance with Clause 9 using a sterile membrane.

Place the membrane on a Petri dish containing WLD.

Repeat this procedure for each flask.

10.3.4 Seeding on M-Green

Aseptically filter the extraction solution prepared in accordance with Clause 9, using a sterile membrane.

Place the membrane on a Petri dish containing M-Green.

Repeat this procedure for each flask.

11 Blank test

Prepare a blank test for each media.

12 Incubation

Invert the WLD and the M-Green dishes and incubate in an incubator (6.2) at 30 °C ± 2 °C for 3 days.

Observe and count the colonies on each plate at least every 24 h.

13 Expression of results

13.1 Determination of the cfu number of bacteria per cork stopper

After the specified incubation period, count the colonies of bacteria on each WLD dish, always referring to the last valid counting.