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Pulp, paper and board — Microbiological examination —

Part 1: Enumeration of bacteria and bacterial spores based on disintegration

Pâte, papier et carton — Analyse microbienne —

Partie 1: Dénombrement des bactéries et des spores bactériennes basé sur la désagrégation

[Revision of second edition (ISO 8784-1:2005)]

ICS 07.100.99; 85.040; 85.060

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Contents

Page

Foreword

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ISO 8784-1 was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*, Subcommittee SC 2, *Test methods and quality specifications for paper and board*.

This third edition cancels and replaces the second edition (ISO 8784-1:2005), which has been technically revised. The second edition was applicable to yeast and mould as well as bacteria. In the revision the content has been divided into two parts, in Part 1 bacteria and bacterial spores are enumerated after disintegration and in Part 2 yeast and mould are enumerated as surface count.

ISO 8784 consists of the following parts, under the general title *Pulp, paper and board — Microbiological examination*:

— *Part 1: Enumeration of bacteria and bacterial spores based on disintegration*

— *Part 2: Enumeration of yeast and mould on surface*[1)]

1) Part 2 is under development and not yet published.

Introduction

This part of ISO 8784, which deals with the microbiological examination of dry market pulp, paper and paperboard, is broadly based on ISO 4833[1], although the conditions are not identical: However, it provides specific amplification where necessary. It is intended for the estimation of colony-forming units, CFU, of aerobic bacteria and bacterial spores.

Because of the exacting techniques required in aseptic procedures, reproducible good quality results can only be ensured by skilled microbiological technicians.

Pulp, paper and board — Microbiological examination —

Part 1: Enumeration of bacteria and bacterial spores based on disintegration

1 Scope

This part of ISO 8784 specifies a method for determining the total number of colony-forming units of bacteria and bacterial spores in dry market pulp, paper and paperboard after disintegration. The enumeration relates to specific media.

2 Normative references

The following referenced documents are indispensable for the application 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 186:2002, *Paper and board — Sampling to determine average quality*

ISO 7213:1981, *Pulps — Sampling for testing*

ISO 638:2008, *Paper, board and pulps — Determination of dry matter content — Oven-drying method*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
bacteria
microscopic, single-celled organisms that possess a prokaryotic type of cell structure, which reproduce by fission and are able to grow under the test conditions specified in this part of ISO 8784

3.2
bacterial spores
highly resistant, dormant structures, e.g. endospores from certain genera of bacteria

3.3
total bacterial count
number of colony-forming units (CFU) of bacteria and bacterial spores formed after incubation in a standard culture medium, under the test conditions specified in this part of ISO 8784

3.4
spore count
number of colony-forming units (CFU) of bacterial spores formed after incubation in a standard culture medium, under the test conditions specified in this part of ISO 8784

4 Principle

This poured plate method involves enumeration of colonies in a standard culture medium. A fibre suspension, prepared from paper, paperboard or pulp samples, is plated in agar. For enumeration of bacterial spores, the fibre suspension is heated for 10 min at 80 °C prior to plating. The plates are incubated at 32 °C for 48 h. The total numbers of bacteria or bacterial spores are enumerated by counting the colonies formed in the agar. Results are expressed as the number of CFU per gram of sample.

5 Culture media and diluents

5.1 General

All substrates and diluents shall be appropriately sterilized. When preparing the culture medium, make sure that the ingredients are completely dissolved by mixing while heating prior to dispensing into suitable containers for sterilization. See ISO/TS 11133-1[2] and ISO/TS 11133-2[3] for quality assurance and guidelines on preparation and production of culture media.

5.2 Water

When water is mentioned in a formula, use distilled water or purified water, see ISO/TS 11133-1[2].

5.3 Culture media for total bacteria count and spore count

Culture medium shall be prepared as follows, or from commercially available dehydrated culture media according to the manufacturer's instructions. Ready-to-use medium may be used when its composition is comparable to that given in this part of ISO 8784. To test the performance of the medium, see ISO/TS 11133-2[3].

Plate count agar (PCA) composition per litre:

Tryptone	5,0 g
Yeast Extract	2,5 g
Dextrose	1,0 g
Agar	15,0 g
Water	1000 ml
Final pH	7,0 ± 0,2

If PCA is not available, Tryptone glucose extract (TGE) agar may be used (see A.3). The use of TGE as an alternative culture medium is acceptable if it gives comparable results as the standard culture medium. The culture medium used shall be stated in the test report (see 12).

5.4 Diluents

Ringer's solution (see A.1) is preferred, although other isotonic solutions may be used. Ringer's tablets are commercially available.

To facilitate the release of cells from the fibres, it is recommended to add 20 µl of Tween 80 (see A.2) per litre to the Ringer's solution prior to sterilization by autoclaving.

The diluent used and if Tween 80 has been added, shall be stated in the test report (see 12).

6 Apparatus and equipment

6.1 General

All laboratory equipment and parts of the equipment in direct contact with the sample and the diluent or the culture medium shall be sterilized.

6.2 List of equipment

6.2.1 Use ordinary microbiological laboratory equipment, and the following.

6.2.2 Suitable wrapping material, e.g. aluminium foil (non coated and inert), ready-to-use envelopes of different sizes or self-closing plastic bags. All of which are commercially available.

6.2.3 Disintegrator, high speed electrical blender with metal (preferably stainless steel) or glass cup that can be sterilized.

NOTE Other homogenizing system with equivalent efficiency may be used.

6.2.4 Incubator, capable of maintaining a constant temperature of 32 °C ± 2 °C.

6.2.5 Petri dishes, having a diameter of 90 mm (standard) or 140 mm to 150 mm (alternative).

6.2.6 Pipettes, of wide-mouth type suitable volume. The width of the mouth must be large enough so that a 1% fibre suspension easily can be drawn into the pipette tip.

NOTE A suitable volume is 10 ml or 50 ml.

6.2.7 Water bath capable of maintaining a temperature of 80 °C ± 2 °C.

6.2.8 Colony-counting equipment or magnifying device with a magnification of at least 1,5 x. The use of an additional lens may be necessary to increase the magnification, up to 10 times, to facilitate the counting of pin-point bacterial colony-forming units and also to ensure that no other particles except bacteria colonies are counted (See 10 Enumeration of colonies).

6.2.9 Balance, with an accuracy of 0,01 g.

6.2.10 Sterilizing unit an autoclave capable of sterilization at 121 °C.

7 Sampling

Make sure that the sampling procedure is performed using aseptic techniques.

If the sample is to represent a lot of paper or paperboard, the sampling shall be in accordance with ISO 186. From each unit of paper or paperboard to be sampled, cut several top layers and discard them to eliminate surface contamination. Use a sterile knife and cut a bunch of sheets. Discard the top sheet.

If the sample is to represent a lot of pulp, the sampling shall be in accordance with ISO 7213. From each unit of dry market pulp to be sampled, discard several top sheets from each bale to eliminate surface contamination.

In other cases, sample a sufficient number of units so that the test material is representative of the paper, the paperboard or the dry market pulp to be tested. In all sampling and examination procedures, make sure that the test material taken is representative of the sample received.

Ideally, a sample should contain at least five sheets, each of them having a minimum size of 200 mm × 250 mm of dry market pulp, paper or paperboard (at least 3 sheets for testing and 2 protective sheets).

After sampling, wrap the unexposed test material in suitable wrapping material (6.2.2).

8 Preparation of the test material

8.1 General

Preferably, conduct the procedure under aseptic conditions. A laminar flow hood is recommended for plating. Weigh the sample outside the hood. Unwrap the test material under aseptic conditions and remove the protective sheets without touching the test sheets.

