

## Pulp, paper and board — Microbiological examination —

### Part 2: Enumeration of bacteria, yeast and mould on surface

*Pâtes, papiers et cartons — Analyse microbienne —*

*Partie 2: Dénombrement des bactéries, des levures et des moisissures en surface*

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## 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](http://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](http://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 of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

A list of all parts in the ISO 8784 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

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## Introduction

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

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# Pulp, paper and board — Microbiological examination

## Part 2: Enumeration of bacteria, yeast and mould on surface

### 1 Scope

This document specifies a method for determining the bacteria, yeast and mould population on the surface of paper and paperboard. The enumeration relates to specific media.

This document is applicable to all kinds of paper and paperboard, to dry market pulp in sheet form and to packaging material.

### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *Paper and board — Sampling to determine average quality*

ISO 7213, *Pulps — Sampling for testing*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

### 3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <https://www.iso.org/obp>

— IEC Electropedia: available at <https://www.electropedia.org/>

#### 3.1

##### **bacteria**

microscopic, single-cell organisms that possess a prokaryotic type of cell structure, which reproduce by fission and are able to grow under the specific test conditions

#### 3.2

##### **yeast**

single-cell fungus, which multiplies mainly vegetatively by budding, able to grow under specific test conditions

#### 3.3

##### **mould**

mycelium forming microfungus, including spores and conidia, able to grow under specific test conditions

#### 3.4

##### **test piece**

portion of the paper or paperboard product which is used in the test to evaluate the number of bacteria, yeast and mould on the surface of the product

### 4 Principle

This method describes a procedure for determining the number of colony-forming units (CFU) of bacteria, yeast and mould transferred from the surface of a sample by making a surface print of the sample on a nutrient agar medium (contact-plate method). Test pieces of paper, paperboard or dry

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market pulp in sheet form are pressed on to the surface of specified agar culture media contact plates for 10 seconds at room temperature. The contact time between the sample and the nutrient agar medium at room temperature is 5 h for bacteria and 20 h for yeast and mould. The test pieces are then removed and contact plates are incubated at  $37\text{ °C} \pm 2\text{ °C}$  for 2 d for bacteria and at  $25\text{ °C} \pm 2\text{ °C}$  for 3 d to 5 d for yeast and mould. At the end of the incubation time, the CFU of bacteria, yeast and mould on the contact plates are counted and the numbers of CFU of bacteria and yeast and mould transferred per  $100\text{ cm}^2$  test surface are calculated.

## **5 Culture media - Contact plates for bacteria, yeast and mould**

Standard RODAC (replicate organism detection and counting) contact plates shall be used. The design of the dish permits the pouring of a raised convex surface of the culture medium for total surface contact of the area being sampled and it may have a grid scored on the base.

Dishes vary in diameter or area, according to the type of surface to be sampled but ready-to-use contact plates with diameter 55 mm are commercially available. Contact plates facilitate easy and reproducible surface microbial testing.

For bacteria, Tryptic Soy Agar (TSA) contact plates shall be used. For yeast and mould, Sabouraud 4 % Dextrose Agar (SDA) with chloramphenicol contact plates shall be used.

Regarding storage and shelf life, follow the recommendations given by the manufacturer.

NOTE The surface area covered by a 55 mm RODAC plate is  $25\text{ cm}^2$ .

## **6 Apparatus**

Use ordinary microbiological laboratory equipment and in particular the following:

### **6.1 General requirements**

The equipment shall be in accordance with ISO 7218.

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

**6.2 Aluminium foil** (non-coated and inert), envelopes, or self-closing plastic bags, for sampling. Samples can be wrapped as such in aluminium foil, in ready-to-use sterile envelopes of different sizes or self-closing plastic bags, of which all are commercially available.

**6.3 Incubator**, capable of maintaining temperatures of  $37\text{ °C} \pm 2\text{ °C}$  and  $25\text{ °C} \pm 2\text{ °C}$ .

**6.4 Petri dishes**, large enough to contain the contact plates.

**6.5 Pair of stainless-steel scissors** sterilised by flaming for cutting sample.

**6.6 Gauge** sterilised by flaming for cutting sample.

**6.7 Forceps or tweezers**, sterilised by flaming.

**6.8 Optional: Colony counting equipment**, fitted with a lens having a magnifying power of at least 1,5 times, to facilitate the counting of small bacteria colonies.

**6.9 Mass or weight of 500 g**.

## **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. 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. From each unit of paper or paperboard to be sampled, cut away several top and



bottom layers and discard them to eliminate surface contamination. Use a sterile knife and cut through several sheets.

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

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

After sampling, wrap the unexposed test material in suitable wrapping material (6.2(6.2)).

## 8 Preparation of the test material

Preferably, the procedure is conducted in aseptic conditions. If available a laminar flow hood is recommended for performing the contact between plates and test pieces.

Unwrap the sample under aseptic conditions and remove the protective sheets without touching the test sheets. With sterilised scissors and a gauge, cut test pieces (60 mm × 60 mm) out from samples by eliminating the edges. These pieces of paper to be tested shall not be touched. Their size shall be superior to the contact plate size and they shall be able to be put down in a Petri dish (regarding the type of samples, 60 mm × 60 mm square test pieces or diameter 80 mm round test pieces for 90 mm diameter Petri dishes).

Five test pieces are necessary for each sample and the total analysed surface shall be at least 100 cm<sup>2</sup>.

If both sides of the test pieces are to be tested, perform two separate experiments. If only one side is tested, specify in the report which side has been tested.

Each test piece shall be kept in sterile Petri dishes for further treatment (side to be tested upward).

NOTE It is possible to cut test pieces of different sizes.

## 9 Procedure

### 9.1 Conditions

This procedure should be carried out in aseptic conditions. The work area should be cleaned with a suitable disinfectant. If available, a laminar flow hood is recommended.

### 9.2 Collection of bacteria, yeast and mould

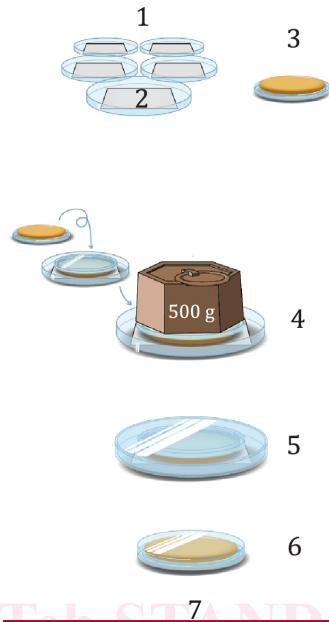
For the determination of bacteria, place the Petri dish containing the test piece on a flat, horizontal surface. Take off the lid of the Petri dish and press the convex agar of the TSA contact plate onto the surface to be investigated, firmly and without any lateral movement against the test surface for 10 s, ensuring an even pressure over the whole plate by using a mass of 500 g.

After a 10 s pressure, remove the mass, replace the lid of the Petri dish and pursue the contact between the contact plate and the test piece at room temperature (23 °C ± 2 °C) for 5 h. After this contact time, remove the contact plate without any lateral movement against the test surface. Replace the lid of the contact plate. Repeat the procedure for each of the five test pieces.

For the determination of yeast and mould, use the same procedure with the SDA chloramphenicol contact plates. After 10 s pressure, remove the weight, replace the lid of the Petri dish and pursue the contact between the contact plate and the test piece at room temperature (23 °C ± 2 °C) for 20 h. After this contact time, remove the contact plate without any lateral movement against the test surface. Replace the lid of the contact plate. Repeat the procedure for each of the five test pieces.

Figure 1 The figure A.1 represents the procedure schematically.

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**Key**

- |   |  |   |  |
|---|--|---|--|
| 1 | Test area: 5 pieces (one per Petri dish)   | 6 | Remove the contact plate, replace the lid and incubate:<br>- 48 h at 37 °C for bacteria<br>- 5 d at 25 °C for yeast and mould  |
| 2 | Test piece 60 x 60 mm  |   |  |
| 3 | Contact plate 55 mm<br>- TSA for bacteria<br>- Sabouraud Chloramphenicol for yeast and mould   |   |  |
| 4 | Contact between the contact plate and a test piece:<br>- 10 s with a 500 g mass  | 7 | Analysed surface area = 125 cm <sup>2</sup><br>5 tests pieces/sample: CFU/125 cm <sup>2</sup> is the sum of colonies on the 5 plates<br>Count colonies and calculate C.F.U (Colony Forming Units) of bacteria, yeasts and moulds per 100 cm <sup>2</sup> |
| 5 | Remove the mass, replace the Petri dish lid, pursue the contact at room temperature:<br>- 5 h for bacteria<br>- 20 h for yeast and mould |   |  |

**Figure 1 — Schematic representation of the procedure**

**9.3 Incubation**

Invert and incubate the contact plates prepared as per 9.2 paragraph 9.2 in an incubator (6.3(6.3)) at 37 °C ± 2 °C for 48 h ± 3 h for bacteria (TSA contact plates) and 25 °C ± 2 °C for 3 d to 5 d for yeast and mould (SDA chloramphenicol contact plate).

**10 Enumeration of the colonies**

After 48 h of incubation, count the number of bacterial colonies on the surface of TSA contact plates.

After 3 d and 5 d of incubation, count the number of yeast and mould colonies on the surface of SDA chloramphenicol contact plates. Retain enumeration after 3 d and 5 d of incubation to avoid the overgrowth of mould that can cover the whole plate inhibiting other colonies from growing. If plates are covered by moulds or if colonies are not growing isolated after 3 d, state it in the test report.