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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

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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).

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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.

Introduction

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 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 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 °C ± 2 °C for 2 d for bacteria and at 25 °C ± 2 °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 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 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 °C ± 2 °C and 25 °C ± 2 °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).

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^2.

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 represents the procedure schematically.