EAST AFRICAN COMMUNITY

EDICT OF GOVERNMENT

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EAS 217-8 (2008) (English): Microbiology of food and animal feeding stuffs — General guidance for enumeration of yeasts and moulds — Part 8: Colony count technique at 25 degrees C

ISO INSIDE

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EAST AFRICAN STANDARD

Microbiology of food and animal feeding stuffs — General guidance for enumeration of yeasts and moulds — Part 8: Colony count technique at 25 degrees C

EAST AFRICAN COMMUNITY

First Edition 2008

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that “Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose”.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

This East African Standard has been revised and aligned to ISO 7954:1987, *Microbiology — General guidance for enumeration of yeasts and moulds — Colony count technique at 25 degrees C*

INTERNATIONAL STANDARD

ISO
7954

First edition
1987-11-01

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ORGANISATION INTERNATIONALE DE NORMALISATION
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Microbiology — General guidance for enumeration of yeasts and moulds — Colony count technique at 25 °C

Microbiologie — Directives générales pour le dénombrement des levures et moisissures — Technique par comptage des colonies à 25 °C

Reference number
ISO 7954 : 1987 (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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 7954 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

Printed in Switzerland

Microbiology — General guidance for enumeration of yeasts and moulds — Colony count technique at 25 °C

0 Introduction

This International Standard is intended to provide general guidance for the microbiological examination of food products not dealt with by existing International Standards and to be taken into consideration by bodies preparing microbiological methods of test for application to foods or to animal feeding stuffs.

Because of the large variety of products within this field of application, these guidelines may not be appropriate for some products in every detail, and for some other products it may be necessary to use different methods.

Nevertheless, it is hoped that in all cases every attempt will be made to apply the guidelines provided as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviation from them in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with these guidelines. In cases where International Standards already exist for the product to be tested, they should be followed, but it is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that eventually the only remaining departures from these guidelines will be those necessary for well-established technical reasons.

1 Scope and field of application

This International Standard gives general guidance for the enumeration of viable yeasts and moulds in products intended for human consumption or feeding of animals by means of the colony count technique at 25 °C.

NOTE — Owing to the nature of yeasts and moulds, the enumeration is subject to certain imprecisions.

2 References

ISO 6887, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218, *Microbiology — General instructions for microbiological examinations.*

3 Definition

For the purpose of this International Standard, the following definition applies.

yeasts and moulds : Micro-organisms which at 25 °C form colonies in a selective medium according to the method specified in this International Standard.

4 Principle

4.1 Preparation of poured plates using a specified selective culture medium and a specified quantity of the test sample if the initial product is liquid, or of an initial suspension in the case of other products.

Preparation of other plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

4.2 Aerobic incubation of the plates at 25 °C for 3, 4 or 5 days.

4.3 Calculation of the number of yeasts and moulds per gram or per millilitre of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

5 Diluent and culture medium

5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the culture medium, dehydrated basic components or a complete dehydrated medium be used. The manufacturer's instructions shall be rigorously followed.

The chemical products used shall be of recognized analytical quality.

The water used shall be distilled or deionized water, free from substances that might inhibit the growth of yeasts and moulds under the test conditions.

Measurements of pH shall be made using a temperature-compensated pH meter (6.4).

If the prepared diluent and culture medium are not used immediately, they shall, unless otherwise stated, be stored in the dark at between 0 and 5 °C, for no longer than 1 month, in conditions which do not produce any change in their composition.

5.2 Diluent

Refer to ISO 6887 and to any International Standard dealing with the product under examination.

5.3 Yeast extract-dextrose-chloramphenicol-agar medium

Table

Component	Quantity
Yeast extract	5 g
Dextrose ($C_6H_{12}O_6$)	20 g
Chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)	0,1 g*
Agar	12 to 15 g**
Water	1 000 ml

* In order to obtain a final concentration of 100 µg/ml of medium.

** According to the manufacturer's instructions.

Dissolve the components in the water by boiling.

If necessary adjust the pH so that after sterilization it is 6,6.

Dispense the agar medium into suitable containers (6.5).

Sterilize at 121 ± 1 °C for 15 min.

NOTE — Chloramphenicol may be replaced by oxytetracycline ($C_{22}H_{30}N_2O_{11}$). In this case, prepare the basic medium as described above, omitting the chloramphenicol, dispense it in quantities of 100 ml and sterilize. Prepare also a 0,1 % (*m/m*) solution of oxytetracycline hydrochloride in water and sterilize by filtration. Just prior to use, add 10 ml of this solution aseptically to 100 ml of the basic medium, which has been previously melted and maintained at 45 °C.

6 Apparatus and glassware

NOTE — Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment, and in particular

6.1 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)** (autoclave either operating separately or being a part of a general apparatus for the preparation and distribution of media).

Sterilize apparatus that will come into contact with the diluent, the culture medium or the sample, particularly plastics apparatus, except for apparatus that is supplied sterile, by one of the following methods :

a) in the oven (6.1) by maintaining it at 170 to 175 °C for not less than 1 h;

b) in the autoclave (6.1) by maintaining it at 121 ± 1 °C for not less than 20 min.

6.2 Incubator, capable of being maintained at 25 ± 1 °C.

6.3 Water-bath, capable of being maintained at 45 ± 1 °C.

6.4 Temperature-compensated pH meter, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.

6.5 Culture bottles or **flasks.**

NOTE — Bottles or flasks with non-toxic metal screw-caps may be used.

6.6 Graduated pipettes, calibrated for bacteriological use only, of nominal capacities 10 and 1 ml, graduated in divisions of 0,5 and 0,1 ml respectively, and with an outflow opening of 2 to 3 mm

6.7 Petri dishes, of diameter 90 to 100 mm.

7 Sampling

Carry out sampling in accordance with the specific International Standard appropriate to the product concerned.

If no specific International Standard exists, it is recommended that agreement be reached on this subject by the parties concerned.

8 Preparation of the test sample

See the specific International Standard appropriate to the product concerned.

If no specific International Standard exists, it is recommended that agreement be reached on this subject by the parties concerned.

9 Procedure

9.1 Test portion, initial suspension and dilutions

Refer to ISO 6887 and to the specific International Standard appropriate to the product concerned.

If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.7). Transfer to each dish, by means of a sterile pipette (6.6), 1 ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products.

9.2.2 Take two further sterile Petri dishes. Transfer, by means of another sterile pipette, 1 ml of the 10^{-1} dilution to each dish (liquid product), or 1 ml of the 10^{-2} dilution (other product).

Repeat the procedure described above using further dilutions if necessary.

9.2.3 Pour about 15 ml of the yeast extract-dextrose-chloramphenicol-agar medium (5.3), previously melted and maintained at 45 ± 1 °C in a water-bath (6.3), from a culture bottle (6.5) into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min.

Carefully mix the inoculum with the medium and allow the mixture to solidify, by leaving the Petri dishes to stand on a cool horizontal surface.

Prepare a control plate, with 15 ml of the medium, to check its sterility.

9.2.4 Invert the plates and place them in the incubator (6.2) at 25 ± 1 °C.

9.3 Interpretation

Count the colonies on each plate after 3, 4 and 5 days of incubation. After 5 days, retain those plates containing fewer than 150 colonies. If parts of the plates are overgrown with moulds, or if it is difficult to count well-isolated colonies, retain the counts obtained after 4 or even 3 days of incubation. In this event, record the incubation period of 3 or 4 days in the test report.

If necessary, carry out a microscopic examination in order to distinguish, according to their morphology, the colonies of yeasts and moulds from colonies of bacteria.

10 Expression of results

10.1 Calculation

10.1.1 Use counts from plates containing fewer than 150 colonies.

10.1.2 The number of yeasts and moulds per gram or per millilitre is equal to

$$\frac{\Sigma C}{(n_1 + 0{,}1\, n_2)\, d}$$

where

ΣC is the sum of the colonies counted on all the plates;

n_1 is the number of plates counted in the first dilution;

n_2 is the number of plates counted in the second dilution;

d is the dilution from which the first counts were obtained (e.g. 10^{-2}).

10.1.3 Round the result obtained in 10.1.2 to two significant figures. When the number to be rounded is 5, with no further significant figures, round the number to give an even figure immediately to the left, for example 28 500 is rounded to 28 000; 11 500 is rounded to 12 000.

10.1.4 The result shall be expressed as a number between 1,0 and 9,9 multiplied by 10^x, where x is the appropriate power of 10.

If there were no colonies on plates from the initial suspension (9.1), if the initial product was solid, the number of yeasts and moulds per gram of product should be reported as fewer than 10.

If there were no colonies on plates from the test sample, if the initial product was liquid (9.1), the number of yeasts and moulds per millilitre of product should be reported as fewer than 1.

10.2 Example of calculation

A yeasts and moulds count gave the following results (two Petri dishes per dilution were incubated) :

10^{-2} dilution : 83 and 97 colonies

10^{-3} dilution : 33 and 28 colonies

$$\frac{\Sigma C}{(n_1 + 0{,}1\, n_2)\, d} = \frac{83 + 97 + 33 + 28}{[2 + (0{,}1 \times 2)] \times 10^{-2}} = \frac{241}{0{,}022}$$

$$= 10\,954$$

Rounding the result as specified in 10.1.3 gives 11 000.

The estimated number of yeasts and moulds per gram or per millilitre is therefore $1{,}1 \times 10^4$.

10.3 Precision

For statistical reasons, in 95 % of cases the confidence levels for this method vary from ± 16 % to ± 52 % [1]. In practice, even larger variations may be observed, in particular between the results obtained by different microbiologists.

11 Test report

The test report shall show the method used, the incubation time and the results obtained, indicating clearly the method of expression used. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

12 Bibliography

[1] COWELL and MORISETTI. *J. Sci. Food Agric.*, 1969 (Vol. 20), p. 573.

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