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Meat and meat products — Enumeration of yeasts and moulds — Colony-count technique

Viandes et produits à base de viande — Dénombrement des levures et moisissures — Technique par comptage des colonies

Reference number
ISO 13681:1995(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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13681 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products*.

Annex A forms an integral part of this International Standard. Annex B is for information only.

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Meat and meat products — Enumeration of yeasts and moulds — Colony-count technique

1 Scope

This International Standard specifies a method for the enumeration of yeasts and moulds in all kinds of meat and meat products, including poultry, by means of a colony-count technique at between 20 °C and 25 °C.

IMPORTANT — ISO 7954[1] recommends chloramphenicol or oxytetracycline as antibiotic. These recommended antibiotics do not, however, sufficiently inhibit Gram-negative microorganisms occurring in meat, especially raw meat. To obtain sufficient inhibition in cases of heavy bacterial contamination, the addition of gentamicin is necessary. Since a combination of chloramphenicol and gentamicin inhibits certain types of yeasts, the alternatively specified antibiotic oxytetracycline is the antibiotic of choice.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3100-2:1988, *Meat and meat products — Sampling and preparation of test samples — Part 2: Preparation of test samples for microbiological examination.*

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218:—[1)], *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

3 Definition

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

3.1 yeasts and moulds: Microorganisms that form colonies within 5 days at between 20 °C and 25 °C under the conditions specified in this International Standard.

4 Principle

4.1 Deep inoculation of poured plates, using a specified selective culture medium contained in Petri dishes, with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Inoculation of other plates, under the same conditions, using decimal dilutions of the initial suspension.

NOTE 1 To distinguish, if necessary, between yeasts and moulds, the use of a surface plate is advisable. Surface plates are also recommended when heat-sensitive yeasts or moulds are expected.

4.2 Aerobic incubation of the plates at between 20 °C and 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.

1) To be published. (Revision of ISO 7218:1985)

5 Dilution fluid, culture medium and reagents

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Dilution fluid

See ISO 6887.

5.3 Agar medium of yeast extract, glucose and oxytetracycline/gentamicin

5.3.1 Base medium

5.3.1.1 Composition

Yeast extract	5 g
Glucose ($C_6H_{12}O_6$)	20 g
Agar	8 g to 18 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

5.3.1.2 Preparation

Dissolve the components in the water by boiling.

If necessary, adjust the pH so that after sterilization it is 6,6 at 25 °C.

Transfer the medium to suitable containers (6.6).

Sterilize for 10 min in the autoclave (6.1) set at 115 °C.

5.3.2 Oxytetracycline solution

5.3.2.1 Composition

Oxytetracycline ($C_{22}H_{30}N_2O_4$)	50 mg
Water	25 ml

5.3.2.2 Preparation

Dissolve the oxytetracycline in the water and sterilize the solution by filtration.

5.3.3 Gentamicin solution

5.3.3.1 Composition

Gentamicin	25 mg [1]
Water	25 ml

1) According to the manufacturer's specification of the real gentamicin content of the powder.

5.3.3.2 Preparation

Dissolve the gentamicin in the water and sterilize the solution by filtration.

5.3.4 Complete medium

Add 5 ml of the oxytetracycline solution (5.3.2) and 5 ml of the gentamicin solution (5.3.3) to each portion of 100 ml of sterile base medium (5.3.1), melted and kept in a water bath (6.4) set at 47 °C.

6 Apparatus and glassware

NOTE 2 Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

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

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

The autoclave can either operate separately or be part of a general apparatus for preparing and distributing media.

See ISO 7218.

6.2 Mixer, working on the principle of a centric rotation of the contents of the tube (e.g. Vortex mixer), and capable of mixing 1 ml or 2 ml of sample in a tube of adequate dimensions with 9 ml or 18 ml of dilution fluid to obtain a homogeneous suspension.

6.3 Incubator, capable of operating at between 20 °C ± 1 °C and 25 °C ± 1 °C.

6.4 Water bath, capable of operating at 47 °C ± 2 °C.

6.5 pH-meter, accurate to within ± 0,1 pH unit at 25 °C.

6.6 Culture bottles or **flasks**

Bottles or flasks with non-toxic metallic screw-caps may be used.

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

6.8 Petri dishes, of diameter 90 mm to 100 mm.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 3100-1[2].

8 Preparation of test sample

Prepare the test sample in accordance with ISO 3100-2.

Start the examination of the pretreated sample as soon as possible. It may be stored, if necessary, at a temperature between 0 °C and + 2 °C, but for no longer than 24 h.

9 Procedure

9.1 Test portion, initial suspension and dilutions

Prepare the initial suspension and dilutions in accordance with ISO 6887.

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.8). Transfer, by means of a sterile pipette (6.7), 1 ml of the test sample to each dish if liquid, or 1 ml of the initial suspension in the case of other products.

9.2.2 Repeat the procedure described in 9.2.1 with the other dilutions.

9.2.3 Pour, from a culture bottle (6.6), about 15 ml of the agar medium of yeast extract, glucose and oxytetracycline/gentamicin (5.3), melted and kept at 47 °C in the water bath (6.4), into each Petri dish. The time elapsing between the end of the preparation of the test sample 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.

9.2.4 Invert the plates and place them in the incubator (6.3) set at between 20 °C and 25 °C.

9.3 Counting and selection of colonies

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, record the counts obtained after 4 (or even 3) days of incubation. In this event, record the incubation period, 3 or 4 days, in the test report.

If necessary, proceed to a microscopic examination in order to distinguish the colonies of yeasts and moulds from colonies of bacteria which are mostly of small size.

10 Expression of results

10.1 General case

Use dishes containing fewer than 150 colonies (see 9.3).

Calculate the number, N, of microorganisms per gram or per millilitre of product, using the following equation:

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

where

ΣC is the sum of all colonies counted on all the dishes retained;

n_1 is the number of dishes retained at the first dilution;

n_2 is the number of dishes retained at the second dilution;

d is the dilution factor corresponding to the first dilution retained (i.e. that with the higher concentration of test sample).

Round off the results calculated to two significant figures. For this, if the last figure is below 5, the preceding figure is not modified; if the last figure is 5 or more, the preceding figure is increased by one unit.

Proceed stepwise until two significant figures are obtained.

Take as the result the number of microorganisms per millilitre (liquid product) or per gram (other products), expressed as a number between 1,0 and 9,9 multiplied by the appropriate power of 10.

EXAMPLE

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

— at the first dilution $\left(10^{-2}\right)$: 83 and 97 colonies;

— at the second dilution $\left(10^{-3}\right)$: 33 and 28 colonies.

Therefore

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

$$= \frac{241}{0{,}022} = 10\,954$$

Rounding the result as specified above gives 11 000 or $1{,}1 \times 10^4$ yeasts and moulds per millilitre or per gram of product.

10.2 Estimation of small numbers

If the two dishes, at the level of the test sample (liquid products) or the initial suspension (other products), contain less than 15 colonies, calculate the arithmetic mean y of the colonies counted on both dishes.

Express the result as follows:

— for liquid products: estimated number of yeasts and moulds per millilitre $N_E = y$

— for other products: estimated number of yeasts and moulds per gram $N_E = y/d$

where d is the dilution factor of the initial suspension.

10.3 No colonies on plates

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

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

11 Precision

For statistical reasons alone, in 95 % of cases the confidence limits of the colony-count technique vary from ± 16 % to ± 52 % (see reference [3]); for colony counts of less than 15 per plate, the confidence limits are given in annex A. In practice, even greater variation may be found, especially among results obtained by different workers.

12 Test report

The test report shall specify

— the method in accordance with which sampling was carried out, if known;

— the method used, including the number of days of incubation;

— the test result(s) obtained; and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

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

Annex A
(normative)

Confidence limits for the estimation of small numbers of colonies

The confidence limits at the 95 % level for the estimation of small numbers, when the number of colonies retained is less than 15, are as follows.

Number of colonies[1]	Number of microorganisms	Confidence limit at 95 % level		Percent of error for the limit[2]	
		Lower	Upper	Lower	Upper
1	1	< 1	3	− 97	+ 457
2	1	< 1	4	− 88	+ 261
3	2	< 1	4	− 79	+ 192
4	2	1	5	− 73	+ 156
5	2	1	6	− 68	+ 133
6	3	1	6	− 63	+ 118
7	4	2	7	− 60	+ 106
8	4	2	8	− 57	+ 97
9	4	2	9	− 54	+ 90
10	5	2	9	− 52	+ 84
11	6	3	10	− 50	+ 79
12	6	3	10	− 48	+ 75
13	6	3	11	− 47	+ 71
14	7	4	12	− 45	+ 68
15	8	4	12	− 44	+ 65
16	8	5	13	− 43	+ 62
17	8	5	14	− 42	+ 60
18	9	5	14	− 41	+ 58
19	10	6	15	− 40	+ 56
20	10	6	15	− 39	+ 54
21	10	6	16	− 38	+ 53
22	11	7	17	− 37	+ 51
23	12	7	17	− 36	+ 50
24	12	8	18	− 36	+ 49
25	12	8	18	− 35	+ 48
26	13	8	19	− 35	+ 47
27	14	9	20	− 34	+ 46
28	14	9	20	− 34	+ 45
29	14	9	21	− 33	+ 44
30	15	10	21	− 32	+ 43

1) Counted on two Petri dishes.

2) Compared to the microorganism count (column 2).

Annex B
(informative)

Bibliography

[1] ISO 7954:1987, *Microbiology — General guidance for enumeration of yeasts and moulds — Colony count technique at 25 °C.*

[2] ISO 3100-1:1991, *Meat and meat products — Sampling and preparation of test samples — Part 1: Sampling.*

[3] COWELL, N.D. and MORISETTI, M.D. *J. Sci. Fd. Agric.*, **20**, 1969, p. 573.

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