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Microbiology of food and animal feeding stuffs — General rules for microbiological examinations

AMENDMENT 1

Microbiologie des aliments — Règles générales pour les examens microbiologiques —
AMENDEMENT 1

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

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Foreword

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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.

Attention is drawn to the possibility that some of the elements of this Amendment may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Amendment 1 to International Standard ISO 7218:1996 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Replace subclause 9.3.4 by the following text.

9.3.4 Counting of colonies

Following the period of incubation stated in the specific standard, count the colonies (total colonies, typical colonies or presumed suspect colonies) for each dish containing less than 300 colonies (or any other number stated in the specific standard).

The different methods of calculation defined in 9.3.5 shall take account of dishes containing 0 colonies if these dishes have been retained.

When counting typical or presumed suspect colonies, the maximum number of all typical or atypical colonies present on a dish shall not exceed 300 (or any other number stated in the specific standard).

NOTE 1 In certain cases, it may be difficult to count the colonies (for example where spreading microorganisms are present). These cases are dealt with in the specific standards.

NOTE 2 When counting typical or presumed suspect colonies, the description of the colonies will possibly be given in the specific standard.

Pages 25 and 26

Replace subclause 9.3.5 by following text.

9.3.5 Expression of results

9.3.5.1 General

9.3.5.1.1 In this subclause, the cases dealt with correspond to the following general cases:

— inoculation of two Petri dishes, 90 mm in diameter, per dilution;

— maximum number for the counting of total colonies is 300 per dish;

— maximum number of all typical and atypical colonies present on a dish when counting typical or presumed suspect colonies is 300 per dish;

— maximum number for the counting of typical or presumed suspect colonies is 150 per dish;

— number of presumed suspect colonies (9.3.5.3) inoculated for identification or confirmation, from each dish retained is 5;

— minimum number of colonies [total colonies, typical colonies or colonies complying with identification or confirmation criteria (9.3.5.3)] on at least one dish is 15.

These figures will be defined in the specific standards.

When dishes with a diameter different from 90 mm are used, the maximum number of colonies shall be increased proportionately to the surface area of the dishes.

9.3.5.1.2 The methods of calculation defined below take account of the cases which occur most frequently when tests are carried out in accordance with good laboratory practice. Rare special cases may occur (for example, significant discrepancy between the number of colonies in two dishes with the same dilution, or a very different ratio to that of the dilution factor between the dishes of two successive dilutions) and it is therefore necessary that results obtained from counting be examined, interpreted or possibly refused by a qualified microbiologist.

9.3.5.2 Method of calculation: General case (counting of total colonies or typical colonies)

For a result to be valid, it is generally considered necessary to count the colonies on at least one dish containing a minimum of 15 colonies [total colonies, typical colonies or colonies complying with identification or confirmation criteria (9.3.5.3)].

Calculate the number N of microorganisms present in the test sample as a weighted mean from two successive dilutions using the following equation:

$$N = \frac{\sum C}{V \times [n_1 + (0{,}1 \times n_2)] \times d} \quad (1)$$

where

$\sum C$ is the sum of the colonies counted on all the dishes retained from two successive dilutions, and where at least one contains a minimum of 15 colonies;

V is the volume of inoculum applied to each dish, in millilitres;

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 [d = 1 when the undiluted liquid product (test sample) is used].

Round off the results calculated to two significant figures. In order to do this, if the third figure is less than 5 do not modify the preceding figure; if the third figure is greater than or equal to 5, increase the preceding figure by one unit.

Take as the result a number preferably between 1,0 and 9,9 multiplied by the appropriate power of 10, or a whole number with two significant figures.

Express the result as follows:

— number N of microorganisms per millilitre (liquid products) or per gram (other products).

EXAMPLE Counting has produced the following results:

— at the first dilution (10^{-2}) retained: 168 and 215 colonies;

— at the second dilution (10^{-3}) retained: 14 and 25 colonies.

$$N = \frac{\sum C}{V \times \left[n_1 + (0{,}1 \times n_2)\right] \times d} = \frac{168 + 215 + 14 + 25}{1 \times \left[2 + (0{,}1 \times 2)\right] \times 10^{-2}} = \frac{422}{0{,}022} = 19\ 182$$

By rounding off the result as recommended above, the number of microorganisms is 19 000 or $1{,}9 \times 10^4$ per millilitre or per gram of product.

9.3.5.3 Method of calculation: Case after identification or confirmation

When the method used requires identification or confirmation, a given number, A (generally 5), of presumed suspect colonies is inoculated from each of the dishes retained for the counting of colonies. After identification or confirmation, calculate, for each of the dishes, the number, a, of colonies complying with identification or confirmation criteria, using the following equation:

$$a = \frac{b}{A} \times C \qquad (2)$$

where

b is the number of colonies complying with identification or confirmation criteria among the inoculated colonies, A;

C is the total number of presumed suspect colonies counted on the dish.

Round off the results calculated to the nearest whole number. In order to do this, if the first figure after the decimal point is less than 5, do not modify the preceding figure; if the first figure after the decimal point is greater than or equal to 5, increase the preceding figure by one unit.

Calculate the number N, N_E *or* N' of identified or confirmed microorganisms present in the test sample, by replacing ΣC by Σa using the formulae provided in 9.3.5.2, 9.3.5.4.1 and 9.3.5.5.3, respectively.

Round off the result as recommended in 9.3.5.2.

Express the result as recommended in 9.3.5.2, 9.3.5.4.1 and 9.3.5.5.3, respectively.

EXAMPLE Counting has produced the following results:

— at the first dilution (10^{-3}) retained: 66 and 80 colonies;

— at the second dilution (10^{-4}) retained: 4 and 7 colonies.

Testing of selected colonies was carried out:

— for 66 colonies, 8 colonies, 6 of which complied with the criteria; hence a = 50;

— for 80 colonies, 9 colonies, 6 of which complied with the criteria; hence a = 53;

— for 7 colonies, 5 colonies, 4 of which complied with the criteria; hence a = 6;

— for 4 colonies, all 4 of which have complied with the criteria; hence a = 4.

$$N = \frac{\sum a}{V \times [n_1 + (0{,}1 \times n_2)] \times d} = \frac{50 + 53 + 6 + 4}{1 \times \left[2 + (0{,}1 \times 2)\right] \times 10^{-3}} = \frac{113}{0{,}002\,2} = 51\ 364$$

By rounding off the result as recommended in 9.3.5.2, the number of microorganisms is 51 000 or $5{,}1 \times 10^4$ per millilitre or per gram of product.

9.3.5.4 Method of calculation: Estimated counts

9.3.5.4.1 Case of two dishes (test sample or initial suspension or first dilution) containing less than 15 colonies

If the two dishes from the test sample (liquid products), or from the initial suspension (other products), or from the first dilution inoculated or retained, contain less than 15 colonies (total colonies, typical colonies or colonies complying with identification or confirmation criteria), calculate the estimated number N_E of microorganisms present in the test sample as an arithmetical mean of the colonies counted on the two dishes using the following equation:

$$N_E = \frac{\sum C}{V \times n \times d} \qquad (3)$$

where

$\sum C$ is the sum of the colonies counted on the two dishes;

V is the volume of inoculum applied to each dish, in millilitres;

n is the number of dishes retained (in this case, $n = 2$);

d is the dilution factor of the initial suspension or of the first dilution inoculated or retained [$d = 1$ when the undiluted liquid product (test sample) is used].

Round off the result as recommended in 9.3.5.2.

Express the result as follows:

— estimated number N_E of microorganisms per millilitre (liquid products) or per gram (other products).

EXAMPLE Counting has produced the following results:

— at the first dilution (10^{-2}) retained, 12 and 13 colonies were counted

$$N_E = \frac{12 + 13}{1 \times 2 \times 10^{-2}} = \frac{25}{0{,}02} = 1\,250$$

By rounding off the result as recommended in 9.3.5.2, the estimated number N_E of microorganisms is 1 300 or $1{,}3 \times 10^3$ per millilitre or per gram of product.

9.3.5.4.2 Case of two dishes (test sample or initial suspension or first dilution) containing no colonies

If the two dishes from the test sample (liquid products), or from the initial suspension (other products), or from the first dilution inoculated or retained, do not contain any colonies, express the result as follows:

— less than $1/d$ of microorganisms per millilitre (liquid products) or per gram (other products);

where d is the dilution factor of the initial suspension or of the first dilution inoculated or retained [$d = 1$ when the undiluted liquid product (test sample) is used].

9.3.5.4.3 Special cases (counting of typical or presumed suspect colonies)

9.3.5.4.3.1 If the number of all typical and atypical colonies for the two dishes containing a first dilution d_1 is greater than 300 (or any other number stated in the specific standard), with visible typical colonies or confirmed colonies and if, for the two dishes of the subsequent dilution d_2 containing less than 300 colonies (or any other number stated in the specific standard) no typical or confirmed colony can be counted, express the result as follows:

— less than $1/d_2$ and more than $1/d_1$ of microorganisms per millilitre (liquid products) or per gram (other products);

where d_1 and d_2 are the dilution factors corresponding to dilutions d_1 and d_2.

EXAMPLE Counting has produced the following results:

— at the first dilution (10^{-2}) retained: more than 300 colonies on each of the two dishes, with typical or confirmed colonies present;

— at the second dilution (10^{-3}) retained: 33 and 35 colonies, with no typical or confirmed colonies present.

The result expressed in microorganisms is less than 1 000 and more than 100 per millilitre or per gram of product.

9.3.5.4.3.2 If the number of all typical and atypical colonies for the two dishes containing a first dilution d_1 is greater than 300 (or any other number stated in the specific standard), without visible typical colonies or confirmed colonies and if, for the two dishes of the subsequent dilution d_2 containing less than 300 colonies (or any other number stated in the specific standard) no typical or confirmed colony can be counted, express the result as follows:

— less than $1/d_2$ of microorganisms per millilitre (liquid products) or per gram (other products);

where d_2 is the dilution factor corresponding to the dilution d_2.

EXAMPLE Counting has produced the following results:

— at the first dilution (10^{-2}) retained: more than 300 colonies on each one of the two dishes, with no typical or confirmed colonies present;

— at the second dilution (10^{-3}) retained: 33 and 35 colonies, with no typical or confirmed colonies present.

The result expressed in microorganisms is less than 100 per millilitre or per gram of product.

9.3.5.5 Method of calculation: Special cases

9.3.5.5.1 When the number of colonies counted (total colonies, typical colonies or presumed suspect colonies) is greater than 300 (or any other number stated in the specific standard) for the two dishes containing a first dilution d_1, with a number of colonies (total colonies, typical colonies or colonies complying with identification or confirmation criteria) of less than 15 for the two dishes of the subsequent dilution d_2:

— if the number of colonies for each of the two dishes containing dilution d_1 is within the 324 to 300 interval (upper part of the confidence interval for a weighted mean equal to 300), use the calculation method for general cases (9.3.5.2);

— if the number of colonies for each of the two dishes containing the dilution d_1 is greater than 324 (upper limit of the confidence interval for a weighted mean equal to 300), only take account of the result of the count of dilution d_2 and then proceed with the estimated count (9.3.5.4), except when referring to a maximum number set at 300 for the counting of colonies, if the latter result is less than 10 (lower limit of the confidence interval for a weighted mean equal to 15) since the difference between the two dilutions is then unacceptable.