IS 5401-1 (2012): Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection and Enumeration of Coliforms, Part 1: Colony Count Technique [FAD 15: Food Hygiene, Safety Management and Other Systems]

BLANK PAGE

IS 5401 (Part 1) : 2002
ISO 4832 : 1991

भारतीय मानक

सूक्ष्मजैविकी — कोलीफार्म की गणना के लिए सामान्य मार्गदर्शन

भाग 1 कोलोनी गणना तकनीक

(पहला पुनरीक्षण)

Indian Standard

MICROBIOLOGY — GENERAL GUIDANCE FOR THE ENUMERATION OF COLIFORMS

PART 1 COLONY COUNT TECHNIQUE

(First Revision)

ICS 07.100.01

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

February 2002

Price Group 3

NATIONAL FOREWORD

This Indian Standard (Part 1) (First Revision) which is identical with ISO 4832 : 1991 'Microbiology — General guidance for the enumeration of coliforms — Colony count technique' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of Food Microbiology Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was originally published in 1969. On a review by the technical committee responsible for formulating standards in this area, it was decided to revise this standard and to align with the ISO Standards on the subject. Accordingly, Part 1 of IS 5401 covers general guidance for enumeration of coliforms by colony count technique, which is identical with ISO 4832 : 1991 and Part 2 of IS 5401 covers general guidance for enumeration of coliforms by most probable number technique, which is identical with ISO 4831 : 1991.

In this adopted standard, certain terminology and conventions are not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard', and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, the following International Standard is referred to. Read in its respective place the following:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 6887 : 1983 Microbiology — General guidance for the preparation of dilutions for microbiological examination	IS 10232 : 1982 Guidelines for the preparation of dilutions for microbiological examination for food	Equivalent

The technical committee responsible for the preparation of this standard has reviewed the provision of ISO 7218 : 1985 'Microbiology — General guidance for microbiological examinations' and has decided that it is acceptable for use in conjunction with this standard.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

MICROBIOLOGY — GENERAL GUIDANCE FOR THE ENUMERATION OF COLIFORMS

PART 1 COLONY COUNT TECHNIQUE

(First Revision)

1 Scope

This International Standard gives general guidelines for the enumeration of coliforms present in products intended for human consumption or feeding of animals, by means of the technique of counting colonies on a solid medium, after incubation at 30 °C, 35 °C or 37 °C, this temperature forming the subject of agreement between the parties concerned.

NOTE 1 The incubation temperature of 30 °C is used when the aim of the enumeration is technological; the temperature of 35 °C or 37 °C is used when the aim of the enumeration is more in the field of public health.

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

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations.*

3 Definition

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

coliforms: Bacteria which, at the specified temperature (i.e. 30 °C, 35 °C or 37 °C, as agreed) form characteristic colonies in crystal violet neutral red bile lactose agar under the test conditions specified in this International Standard.

4 Principle

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

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

4.2 Incubation of the plates at 30 °C, 35 °C or 37 °C (as agreed) for 24 h.

4.3 Calculation of the number of coliforms per millilitre or per gram of sample from the number of characteristic colonies obtained in the plates chosen (see 10.1).

5 Culture medium and dilution fluid

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Dilution fluid

See ISO 6887 and the specific International Standard dealing with the product under examination.

5.3 Solid selective medium: crystal violet neutral red bile lactose (VRBL) agar

Composition

peptone	7 g
yeast extract	3 g
lactose	10 g
sodium chloride	5 g
bile salts	1,5 g
neutral red	0,03 g
crystal violet	0,002 g
agar	12 g to 18 g[1)]
water	1 000 ml

Preparation

Proceed as follows in order to conserve the selective power and specificity of the medium.

Thoroughly mix the components or the dehydrated complete medium in the water and leave to stand for several minutes. Adjust the pH so that, after boiling, it is 7,4 at 25 °C. Bring to the boil, stirring from time to time.

Allow to boil for 2 min. Immediately cool the medium in the water-bath (6.5) set at 45 °C.

Avoid overheating the medium, heating it for too long or reheating it. Consequently, do not sterilize in the autoclave, and check the sterility of the medium at the time of use (see 9.2.2).

Use the medium within 3 h of its preparation.

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).

See ISO 7218.

6.2 Incubator, capable of operating at 30 °C ± 1 °C, 35 °C ± 1 °C or 37 °C ± 1 °C.

6.3 Petri dishes, made of glass or plastic, of diameter 90 mm to 100 mm.

6.4 Total delivery pipettes, having a nominal capacity of 1 ml.

6.5 Water-bath, or similar apparatus, capable of operating at 45 °C ± 0,5 °C.

6.6 Colony counting equipment, consisting of an illuminated base and a mechanical or electronic digital counter.

6.7 pH meter, accurate to ± 0,1 pH unit at 25 °C.

7 Sampling

Sampling shall have been carried out in accordance with 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.

8 Preparation of the test sample

Prepare the test sample in accordance with the specific International Standard dealing with 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 Procedure

9.1 Test portion, initial suspension and dilutions

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

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.3). Using a sterile pipette (6.4), transfer to each dish 1 ml of the test sample, if the product is liquid, or 1 ml of the initial suspension in the case of other products.

Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1 ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products.

Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.

1) According to the gel strength of the agar.

9.2.2 Pour about 15 ml of the VRBL medium (5.3), at 45 °C ± 0,5 °C, 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 (5.3) is poured into the dishes shall not exceed 15 min.

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

Also prepare a control plate, with 15 ml of the medium for checking its sterility.

9.2.3 After complete solidification, pour about 4 ml of the VRBL medium (5.3), at 45 °C ± 0,5 °C, on to the surface of the inoculated medium. Allow to solidify as described above.

9.2.4 Invert the prepared dishes and incubate them in the incubator set at 30 °C, 35 °C or 37 °C (as agreed) for 24 h ± 2 h.

9.3 Counting of the colonies

After the specified period of incubation (see 9.2.4), count, using the colony counting equipment (6.6), the characteristic coliform colonies in each dish containing not more than 150 colonies[2] whether characteristic or not.

NOTE 3 After incubation for 24 h, characteristic colonies are purplish red colonies having a diameter of 0,5 mm or greater and sometimes surrounded by a reddish zone of precipitated bile.

10 Expression of results

10.1 Method of calculation

10.1.1 General case — Dishes containing between 15 and 150 characteristic colonies

Retain dishes containing not more than 150 characteristic colonies at two consecutive dilutions. It is necessary that one of these dishes contains at least 15 characteristic colonies.

Calculate the number N of coliforms per millilitre or per gram of product, depending on the case, using the following equation:

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

where

$\sum C$ is the sum of the characteristic colonies counted on all the dishes retained;

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

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

d is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures.

Take as the result the number of coliforms per millilitre or per gram of product, expressed as a number between 1,0 and 9,9 multiplied by 10^x, where x is the appropriate power of 10.

EXAMPLE

A coliform count at 30 °C gave the following results:

— at the first dilution retained (10^{-2}): 83 and 97 characteristic colonies

— at the second dilution retained (10^{-3}): 13 and 8 characteristic colonies

$$N = \frac{\sum C}{(n_1 + 0{,}1 n_2) d} = \frac{83 + 97 + 13 + 8}{(2 + 0{,}1 \times 2) \times 10^{-2}} = \frac{201}{0{,}022} = 9136$$

Rounding the result as specified above gives 9 100 or 9,1 × 10^3 coliforms per millilitre or per gram of product.

10.1.2 Case where each dish contains less than 15 characteristic colonies

If each of the dishes retained contains less than 15 characteristic colonies, calculate the estimated number N_E of coliforms using the equation given in 10.1.1.

EXAMPLE

A coliform count at 30 °C gave the following results:

— at the 10^{-4} dilution: 140 and 145 colonies, of which 5 and 3 colonies respectively were characteristic

2) Above this number there is a risk that coliform colonies will have an atypical appearance.

— at the 10^{-5} dilution: 11 and 8 colonies, of which 0 and 1 colonies respectively were characteristic

$$N_E = \frac{5 + 3 + 0 + 1}{(2 + 0{,}1 \times 2) \times 10^{-4}}$$

$$= \frac{9}{2{,}2 \times 10^{-4}} = 40000$$

Rounding the result as specified in 10.1.1 gives 4,0 $\times 10^4$ coliforms per millilitre or per gram of product.

10.1.3 Estimation of small numbers

If the two dishes, corresponding to the test sample (liquid products) or the initial suspension (other products), contain less than 15 characteristic colonies, report the result as follows:

— less than 15 coliforms per millilitre (liquid products);

— less than $15 \times 1/d$ coliforms per gram (other products), where d is the dilution factor of the initial suspension.

10.1.4 No characteristic colonies

If the two dishes, corresponding to the test sample (liquid products) or the initial suspension (other products), contain no characteristic colonies, report the result as follows:

— less than 1 coliform per millilitre (liquid products);

— less than $1 \times 1/d$ coliform per gram (other products), where d is the dilution factor of the initial suspension.

10.2 Precision

10.2.1 Dishes containing between 15 and 150 characteristic colonies (see 10.1.1)

For statistical reasons alone, in 95 % of cases the confidence limits of this method vary from $\pm$ 16 % to $\pm$ 52 % (Cowell and Morisetti, *J. Sci. Fd. Agric.*, 1969, Vol. 20, p. 573). In practice, even greater variation may be found especially among results obtained by different microbiologists.

10.2.2 Each dish contains less than 15 characteristic colonies (see 10.1.2)

Refer to table A.1. To obtain the confidence limits, multiply the lower and upper limits given by $1/d$, where d is the dilution factor.

10.2.3 Estimation of small numbers (see 10.1.3)

The confidence limits for the estimation of small numbers of coliforms are given in table A.1.

11 Test report

The test report shall specify the method used, the aim (technological or public health) of the test and the temperature chosen, and the results 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 results.

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 characteristic colonies on dishes retained is less than 15, are given in table A.1.

Table A.1

Number of coliforms	Confidence limits at the 95 % level	
	lower	upper
1	< 1	2
2	< 1	4
3	< 1	5
4	1	6
5	2	9
6	2	10
7	2	12
8	3	13
9	4	14
10	4	16
11	5	18
12	6	19
13	7	20
14	7	21
15	8	23

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act,* 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 46 (1103).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002
Telephones : 323 01 31, 323 33 75, 323 94 02

Telegrams: Manaksanstha
(Common to all offices)

Regional Offices :

Telephone

Central : Manak Bhavan, 9 Bahadur Shah Zafar Marg
NEW DELHI 110002 — 323 76 17, 323 38 41

Eastern : 1/14 C. I. T. Scheme VII M, V. I. P. Road, Kankurgachi
KOLKATA 700 054 — 337 84 99, 337 85 61, 337 86 26, 337 91 20

Northern : SCO 335-336, Sector 34-A, CHANDIGARH 160 022 — 60 38 43, 60 20 25

Southern : C. I. T. Campus, IV Cross Road, CHENNAI 600 113 — 254 12 16, 254 14 42, 254 25 19, 254 13 15

Western : Manakalaya, E9 MIDC, Marol, Andheri (East)
MUMBAI 400 093 — 832 92 95, 832 78 58, 832 78 91, 832 78 92

Branches : AHMADABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. NALAGARH. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM.

Printed at New India Printing Press, Khurja, India