BHUTAN STANDARD

METHODS FOR YEAST AND MOULD COUNT OF FOODSTUFFS AND ANIMAL FEEDS

ICS 07.100.30

BHUTAN STANDARD BUREAU
The National Standards Body of Bhutan
THIMPHU

XXXX, 2019 Price Group A

BTS 282: XXXX IS 5403: 1999

NATIONAL FOREWORD

This Bhutan Standard which is identical with IS 5403: 1999 METHODS FOR YEAST AND MOULD COUNT OF FOODSTUFFS AND ANIMAL FEEDS Standard issued by the Bureau of Indian Standards was adopted by Bhutan Standards Bureau by Food and Agriculture Technical Committee (TC 02) and approved by the Bhutan Standards Bureau Board (BSB Board) on xxxx, 2019.

The text of the IS Standard has been approved as suitable for publication as Bhutan Standard without deviation. Certain conventions are however, not identical to those used in Bhutan Standard.

Attention is particularly drawn to the following:

a) Where the words “IS Standard” appear referring to this standard, they should be read as “Bhutan Standard”.

b) Wherever page numbers are quoted, they are “IS Standard” page numbers.

IS 5403 : 1999
(Reaffirmed 2005)

भारतीय मानक

खाद्य पदार्थ और पशु-आहार के खमीर और फफूंदी गणन के लिए पद्धति

(पहला पुनरीक्षण)

Indian Standard

METHOD FOR YEAST AND MOULD COUNT OF FOODSTUFFS AND ANIMAL FEEDS

(First Revision)

ICS 07.100.30

BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

May 1999

Price Group 2

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Microbiology Sectional Committee had been approved by the Food and Agriculture Division Council.

Microbiological examination of food provides reliable information regarding its quality, the sanitary conditions under which the food was processed, and the effectiveness of the method of preservation. Keeping this in view, a series of Indian Standards on microbiological analysis has been formulated and it is expected that adoption of these standards would help in achieving uniform microbiological assessment of the foodstuffs that is accepted all over the country. Besides, this would facilitate the interpretation and comparison of results.

High yeast and mould count in food products is not desirable and indicates improper plant sanitation control, improper packing and faulty storage as the responsible factors while manufacturing the products.

This standard, which was originally published in 1969 is being revised in order to align with ISO 7954 : 1987 'Microbiology — General guidance for enumeration of yeasts and moulds — Colony count technique at 25°C'. Owing to the nature of yeasts and moulds, the enumeration is subject to certain imprecisions.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD FOR YEAST AND MOULD COUNT OF FOODSTUFFS AND ANIMAL FEEDS

(First Revision)

1 SCOPE

This standard specifies the method for viable yeast and mould count in products intended for human consumption or feeding of animals by means of the colony count technique at 25°C.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
5404 : 1984	Code of practice for handling of samples for microbiological analysis (*first revision*)
6850 : 1973	Agar, microbiological grade
7004 : 1973	Yeast extract, microbiological grade
10232 : 1982	Guidelines for preparation of dilutions for microbiological examination of food

3 DEFINITION

For the purpose of this standard, the following definition shall apply.

3.1 Yeasts and Moulds

Micro-organisms which at 25°C form colonies in a selective medium according to the method specified in this 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 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and reagent grade water (*see* IS 1070) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test results.

6 SAMPLING

6.1 For microbiological examination, the sample should be handled asceptically and it should be truly representative of the lot. For this purpose IS 5404 shall be followed.

7 DILUTIONS AND CULTURE MEDIUM

7.1 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 (IS 1070), free from substances that might inhibit the growth of yeasts and moulds under the test conditions.

Measurements of *p*H shall be made using a temperature-compensated *p*H meter.

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.

7.2 Dilutions

For preparing required dilutions, the guidelines given in IS 10232 shall be followed.

7.3 Yeast Extract-Dextrose-Chloramphenicol-Agar Medium

The medium shall have the components as given in Table 1.

Table 1 Composition of Medium

Component	Quantity
(1)	(2)
Yeast extract (*see* IS 7004)	5 g
Dextrose ($C_6H_{12}O_6$)	20 g
Chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)	0.1 g[1]
Agar (*see* IS 6850)	12 to 15 g[2]
Water	1 000 ml

[1] In order to obtain a final concentration of 100 µg/ml of medium.

[2] According to the manufacturer's instructions.

7.3.1 Dissolve the components in the water by boiling. If necessary adjust the *p*H so that after sterilization it is 6.6. Dispense the agar medium into suitable containers. 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 percent (*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.

8 APPARATUS AND GLASSWARE

Usual microbiological laboratory equipment, and in particular following.

8.1 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 (**8.1**) by maintaining it at 170 to 175°C for not less than 1 h;

b) In the autoclave (**8.1**) by maintaining it at 121 ± 1°C for not less than 20 min.

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

8.2 Incubator

Capable of being maintained at 25 ± 1°C.

8.3 Water-Bath

Capable of being maintained at 45 ± 1°C.

8.4 Temperature Compensated *p*H Meter

Having an accuracy of calibration of ± 0.1 *p*H unit at 25°C.

8.5 Culture Bottles or Flasks

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

8.6 Graduated Pipettes

Calibrated for bacteriological use only, of nominal capacities 10 ml and 1 ml, graduated in divisions of 0.5 ml and 0.1 ml respectively, and with an outflow opening of 2 to 3 mm.

8.7 Petri Dishes

Of diameter 90 to 100 mm.

9 PROCEDURE

9.1 Inoculation and Incubation

9.1.1 Take two sterile petri dishes (**8.7**). Transfer to each dish, by means of a sterile pipette (**8.6**), 1 ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products.

9.1.2 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.

9.1.3 Pour about 15 ml of the yeast extract-dextrose-chloramphenicol-agar medium (**7.3**), previously melted and maintained at 45 ± 1°C in a water-bath (**8.3**), from a culture bottle (**8.5**) 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.

9.1.4 Invert the plates and place them in the incubator (**8.2**) at 25 ± 1°C.

9.2 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.

9.3 Generally, it is desirable to differentiate between moulds and yeasts. It is advisable to examine the plates at the end of three days for yeast colonies as they are likely to be overgrown by mould growth. Make a separate count of the yeast colonies, which usually will be characterized as smooth, moist, elevated or surface colonies. After counting the typical yeast colonies, count the mould colonies. Mould colonies are easily recognized by their profuse growth of hyphae. If only yeast counts are required, add 0.25 percent of sterile sodium propionate solution to the plate at the time of pouring to inhibit the growth of moulds.

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 = the sum of the colonies counted on all the plates;

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

n_2 = the number of plates counted in the second dilution; and

d = the dilution from which the first counts were obtained (for example, 10^{-1}).

10.1.3 Round the result obtained in **10.1.2** to two significant figures. The result shall be expressed as a number between 1.0 and 9.9 multiplied by $10x$, where x is the appropriate power of 10.

If there were no colonies on plates from the initial suspension, 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, the number of yeasts and moulds per millilitre of product should be reported as fewer than 1.

10.2 Example of Calculation

A yeast and mould 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

$$\frac{C}{(n_1 + 0.1\, n_2)d} = \frac{83 + 97 + 33 + 28}{[2 + (0.1 \times 2)] \times 10^{-2}} = \frac{241}{0.022}$$

$$= 10\,954$$

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 1.1×10^4.

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.

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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 Handbook' and 'Standards : Monthly Additions'.

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