*भारतीय मानक*

***Indian Standard***

**IS 7590 : 2023**

# जिलेटिन, माइक्रोबायोलॉजिकल ग्रेड — विशिष्टि

*( पहला पुनरीक्षण )*

# Gelatin, Microbiological Grade — Specification

*( First Revision )*

ICS 07.100.99



भारतीय मानक ब्यूरो
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Food Microbiology Sectional Committee, FAD 31

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.

Unless the ingredients used in media for microbiological work are of uniform quality, the results obtained would be erroneous and would be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological work at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiology and providing guidance to the indigenous manufacturers regarding the quality of various ingredients, it has been decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological work.

Gelatin is obtained by partial hydrolysis of collagen derived from the skin, white connective tissues and bones of animals. Gelatin, food-grade, is widely used as an emulsifying and thickening agent in various food products. This is covered by IS 5719. Gelatin conforming to lesser number of requirements but passing those mentioned in this standard can be used as an ingredient for microbiological media, for solidification of nutrient broth where temperature of incubation is not more than 20 °C to 25 °C and for the culture of organisms in dairy and milk products. It is also used as a diagnostic reagent for determining proteolysis by liquefaction of nutrient gelatin.

This standard was first published in 1973. The first revision of the standard has been brought out to incorporate following modifications keeping in view the technological advancements in this area along with the editorial changes to align it in the latest style and format of Indian Standard:

a) Modifications have been made in the physical state and colour of the product in **3.1** under ‘description’;
b) *Salmonella* has been replaced with *Salmonella* Typhi in **3.6**;
c) Gelatin liquefaction test at Annex B has been re-written making it easier to understand; and
d) Requirement of ‘Heavy metal’ in the Table 1, Sl No. (v) has been replaced with ‘Lead’, as ‘Heavy metal’ comprises of metals other than lead and the method given is actually leading to the determination of lead only. The method of test has also been changed in line with the method for lead in other standards of microbiological media ingredients.

The composition of the committee responsible for the formulation of this standard is listed in Annex C.

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 : 2022 ‘Rules for rounding off numerical values (*second revision*)’. 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*

# GELATIN, MICROBIOLOGICAL GRADE — SPECIFICATION

*( First Revision )*

## 1 SCOPE

This standard prescribes requirements and methods of sampling and test for gelatin, microbiological grade.

## 2 REFERENCES

The standards listed below contain provisions which, through reference in this text, constitute provisions 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 these standards listed below:

| *IS No.* | *Title* |
|---|---|
| IS 1070 : 1992 | Reagent grade water — Specification (*third revision*) |
| IS 6851 : 1973 | Specification for meat extract, microbiological grade |
| IS 6853 : 1973 | Specification for peptone, microbiological grade |
| IS 6854 : 1973 | Methods of sampling and test for ingredients used in media for microbiological work |

## 3 REQUIREMENTS

### 3.1 Description

Gelatin, microbiological grade shall be white to yellowish powder or granules or flakes. It shall not contain any preservatives which will inhibit the growth of microorganisms.

### 3.2 Solubility

The material shall be insoluble in cold water but shall swell and soften when immersed in it. It shall be soluble in hot water forming a jelly on cooling. It shall dissolve in acetic acid and in hot mixture of glycerol and water. It shall be insoluble in 95 percent alcohol, in chloroform and in ether solvent.

**3.3** A hot solution of the material (1 in 40) shall be free from any disagreeable odour and shall be only slightly opalescent when observed in a layer of 2 cm thickness.

**3.4** A dilute aqueous solution of the material shall pass the identification test given in Annex A.

**3.5** The material shall form a suitable gel with agar. The surface of the gel shall not show any physical distortion (scum-like surface). A gel obtained from 15 percent gelatin shall be firm and media containing this proportion of gelatin should withstand autoclaving at 115 °C for 15 min without loss of gel strength.

**3.6** The material shall pass the test for gelatin liquefaction prescribed in Annex B. When tested on solid medium containing nutrient broth and gelatin, the gelatin should be liquefied by *Proteus vulgaris* at 22 °C and shall be not liquefied by *Salmonella* Typhi.

**3.7** It shall also conform to the requirements given in Table 1.

## 4 PACKING, MARKING AND STORAGE

### 4.1 Packing

The material shall be securely packed in well-filled wide mouth containers with tightly fitting lids.

### 4.2 Storage

The material shall be stored in a cool and dry place.

### 4.3 Marking

Each container shall be marked legibly to give the following information:

a) Name of the material including the words ‘Microbiological Grade’;
b) Name and address of the manufacturer;
c) Minimum net quantity; and
d) Batch and/or code number.

**4.3.1** *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provision of

*Bureau of Indian Standards Act*, 2016 and Rules and Regulation framed thereunder and the product(s) may be marked with the Standard Mark.

## 5 SAMPLING

The representative samples of the material shall be drawn according to the method prescribed in IS 6854.

## 6 TESTS

**6.1** Tests shall be carried out by the methods prescribed in **3** and in col (4) of Table 1.

### 6.2 Quality of Reagents

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

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

**Table 1 Requirements for Gelatin, Microbiological Grade**
(*Clause* 3.7)

| Sl No. | Characteristic | Requirement | Method of Test, Ref to Clause No. of IS 6854 |
|---|---|---|---|
| (1) | (2) | (3) | (4) |
| i) | Moisture, percent by mass, *Max* | 15 | **4** |
| ii) | Total ash, percent by mass, *Max* | 3 | **6** |
| iii) | Nitrogen (on dry basis), percent by mass, *Min* | 15 | **9** |
| iv) | Arsenic (as As), mg/kg, *Max* | 2 | **12** |
| v) | Lead, mg/kg, *Max* | 50 | **14** |

## ANNEX A

(*Clause* 3.4)

## IDENTIFICATION TEST

### A-l PROCEDURE

**A-1.1** To an aqueous solution (1 in 100) of gelatin, add trinitrophenol solution (1 g anhydrous material dissolved in 100 ml water) or a solution of potassium dichromate (1 in 15) previously mixed with one-fourth its volume of dilute hydrochloric acid. A pale yellow precipitate should be formed.

**A-1.2** To an aqueous solution (1 in 5 000) of gelatin, add freshly prepared tannic acid solution (1 g dissolved in 1 ml alcohol and diluted with water to 10 ml). Turbidity should be produced.

## ANNEX B

(*Clause* 3.6)

## GELATIN LIQUEFACTION TEST

### B-1 STRAINS

*Proteus vulgaris* and *Salmonella* Typhi

### B-2 MEDIA AND REAGENT

### B-2.1 Nutrient Agar

**B-2.1.1** *Composition*

| *Ingredients* | *Grams/Litre* |
|---|---|
| Peptone | 5 |
| Sodium chloride | 5 |
| Beef Extract | 1.5 |
| Yeast Extract | 1.5 |
| Agar | 15 |

**B-2.1.2** *Preparation*

Suspend 28.0 g media in 1 000 ml of purified/distilled water. Adjust final *p*H 7.4 ± 0.2 at 25 °C. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 103 kPa (15 lbs) pressure (121 °C) for 15 min. Cool to 45 ° C to 50 °C. Mix well and pour into sterile petri plates.

### B-2.2 Nutrient Gelatin

**B-2.2.1** *Composition*

| *Ingredients* | *Grams/Litre* |
|---|---|
| Peptic digest of animal tissue | 5 |
| Meat extract | 3 |
| Gelatin | 120 |

**B-2.2.2** *Preparation*

Suspend 158 g media in 1 000 ml of warm (50 °C) water. Adjust final *p*H 7.0 ± 0.2 at 25 °C. Heat to 50 °C to dissolve the medium completely. Distribute in approximately 6 ml amounts into clean test tubes of size approximately 12 cm × 2 cm and then sterilize by autoclaving at 103 kPa (15 lbs) pressure (121 °C) for 12 min. Special care is to be taken to ensure that the medium is not overheated.

### B-2.3 Gelatin Agar

**B-2.3.1** *Composition*

| *Ingredients* | *Grams/Litre* |
|---|---|
| Gelatin | 30 |
| Tryptone | 10 |
| Sodium chloride | 10 |
| Agar | 15 |

**B-2.3.2** *Preparation*

Suspend 65.0 g media in warm preheated 1 000 ml purified/distilled water. Adjust final *p*H 7.2 ± 0.2 at 25 °C. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 103 kPa (15 lbs) pressure (121 °C) for 15 min. Cool to 45 °C to 50 °C. Mix well and distribute into sterilized glass petriplates of size approximately 10 cm diameter.

**B-2.4 Acid Mercuric Chloride Solution**

Mix 12.0 g of mercuric chloride in 80.0 ml distilled water. Add 16.0 ml of concentrated HCl and shake well until solution is complete.

**B-3 METHOD**

Any of the two methods may be used:

**B-3.1** Using overnight growths of *P. vulgaris* and *S.* Typhi on nutrient agar (*see* **B-2.1**), inoculate, as stab cultures with a straight wire, individual tubes of nutrient gelatin medium (*see* **B-2.2**), separately, with both the bacterial strains. Incubate the tube containing *P. vulgaris* at 22 °C and the tube containing *S.* Typhi at 37 °C. Observe for liquefaction of the gelatin daily up to 7 days in the cultures of *P. vulgaris* and up to 14 days in the cultures of *S.* Typhi. Uninoculated tubes of nutrient gelatin medium incubated parallel with each set of uninoculated medium serves as control. Observe the presence or absence of liquefaction of gelatin by keeping the incubated tubes in an ice-bath or refrigerator for 2 h to 3 h to allow the gelatin medium in the uninoculated tubes to become firm.

**B-3.2** Using overnight growths of *P. vulgaris* and *S.* Typhi on nutrient agar (*see* **B-2.1**), inoculate individual petri plate of gelatin agar medium (*see* **B-2.3**), separately, with both the bacterial strains. Incubate the petri plate containing *P. vulgaris* at 22 °C and that containing *S.* Typhi at 37 ºC, both for 7 days. To observe liquefaction of gelatin, flood the surface with 5 ml to 10 ml acid mercuric chloride solution. Clear zone around the colonies of bacteria indicate areas of gelatin hydrolysis.

**ANNEX E**

(*Foreword*)

**COMMITTEE COMPOSITION**

Food Microbiology Sectional Committee, FAD 31

| *Organization* | *Representative(s)* |
|---|---|
| ICAR - Indian Veterinary Research Institute, Izzatnagar, Bareilly | DR KIRAN N. BHILEGAONKAR **(*Chairperson*)** |
| CSIR - Central Food Technological Research Institute, Mysuru | DR ALOK K. SRIVASTAVA<br>DR ASHA MARTIN (*Alternate*) |
| CSIR - Institute of Microbial Technology, Chandigarh | DR SURESH KORPOLE<br>DR P. ANIL KUMAR (*Alternate*) |
| Central Research Institute, Kasauli | DR YASHWANT KUMAR<br>DR SUBHADIP MAHAPATRA (*Alternate*) |
| Defence Food Research Laboratory, Mysuru | DR JOSEPH KINGSTON<br>DR BALAKRISHNA *(Alternate*) |
| Export Inspection Council of India, New Delhi | SHRI WASI ASGHAR<br>SHRI ANGSHUMAN SAHA (*Alternate*) |
| HiMedia Laboratories Pvt Ltd, Mumbai | DR RAHUL G. WARKE<br>DR GIRISH B. MAHAJAN (*Alternate*) |
| ICAR - Central Institute for Fisheries Technology, Kochi | DR SATYEN KUMAR PANDA<br>DR B. MADHUSUDAN RAO (*Alternate*) |
| ICAR - Indian Veterinary Research Institute, Izzatnagar, Bareilly | DR TRIVENI DUTT<br>DR D. K. SINGH (*Alternate*) |
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| Marine Products Export Development Authority, Kochi | DR SREENATH P. G.<br>SHRI V. VINOD (*Alternate*) |
| Merck Life Sciences Pvt Ltd, Mumbai | SHRIMATI SUJATA SAINDANE<br>SHRI SACHIN MALI (*Alternate*) |
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| National Dairy Development Board, Anand | SHRI S. D. JAISINGHANI<br>DR JITENDER SINGH (*Alternate* I)<br>DR NAVEEN KUMAR (*Alternate* II) |
| National Institute of Cholera and Enteric Diseases, Kolkata | DR SHANTA DUTTA<br>DR ASISH K. MUKHOPADHYAY (*Alternate*) |
| National Institute of Food Technology Entrepreneurship and Management, Sonipat | DR NEETU KUMRA TANEJA (*Alternate*) |

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SHRIMATI VARSHA GUPTA
SCIENTIST 'D'/JOINT DIRECTOR
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Panel responsible for Review of Indian Standards related to Microbiological Media Ingredients, FAD 31 : Panel 3

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