इंटरनेट मानक

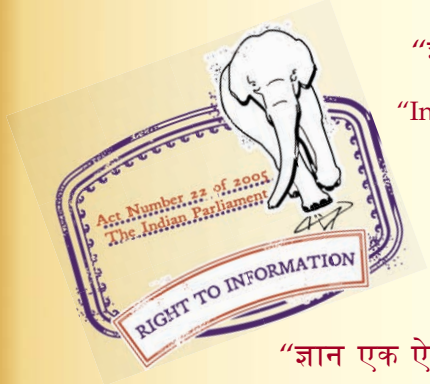
Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 1622 (1981): Methods of sampling and microbiological examination of water [CHD 32: Environmental Protection and Waste Management]



"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

IS : 1622 - 1981
(Reaffirmed 1996)

Indian Standard

METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

Fourth Reprint FEBRUARY 2003

UDC 663.63 : 543.39

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

Gr 10

October 1982

Indian Standard

METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

Water Sectional Committee, CDC 26

Chairman	*Representing*
DR NILAY CHAUDHURI	Central Board for the Prevention & Control of Water Pollution, New Delhi
Members	
MEMBER-SECRETARY (*Alternate* to Dr Nilay Chaudhuri)	
SHRI J. S. CHAUDHRI	Haryana State Board for the Prevention & Control of Water Pollution, Chandigarh
SHRI H. G. GARG (*Alternate*)	
CHIEF WATER ANALYST, KING INSTITUTE, MADRAS	Director of Public Health, Government of Tamil Nadu, Madras
SHRI J. D. CRUZ	Delhi Water Supply & Sewage Disposal Undertaking, New Delhi
SHRI K. R. SAHU (*Alternate*)	
DEPUTY DIRECTOR (CHEM), RDSO, LUCKNOW	Railway Board (Ministry of Railways)
CHEMIST & METALLURGIST, WESTERN RAILWAY, BOMBAY (*Alternate*)	
SHRI M. V. DESAI	Indian Chemical Manufacturers' Association, Calcutta
SHRI MANGAL SINGH (*Alternate*)	
SHRI O. L. DUGGAL	Ballarpur Industries Ltd, Ballarpur
SHRI R. VARADHAN (*Alternate*)	
SHRI B. K. DUTTA	Ion Exchange (India) Ltd, Bombay
SHRI N. RAMACHANDRAN (*Alternate*)	
SHRI R. C. DWIVEDI	U. P. Water Pollution Prevention & Control Board, Lucknow
SHRI S. P. SAXENA (*Alternate*)	
SHRI R. S. EKBOTE	Hindustan Organic Chemicals Ltd, Rasayani
DR M. I. GURBAXANI	The Tata Iron & Steel Co Ltd, Jamshedpur
SHRI C. P. JAIN	Central Electricity Authority, New Delhi
SHRI J. JHA (*Alternate*)	
SHRI K. K. KAMATH	Kerala State Board for Prevention & Control of Water Pollution, Trivandrum
SHRI J. D. JOYSINGH (*Alternate*)	
DR P. K. MATHUR	Bhabha Atomic Research Centre, Bombay
PROF R. S. MEHTA	Gujarat Water Pollution Control Board, Gandhinagar
SHRI M. D. DAVE (*Alternate*)	
MEMBER-SECRETARY	Maharashtra Prevention of Water Pollution Board, Bombay
SHRI S. K. MITRA	West Bengal Prevention & Control of Water Pollution Board, Calcutta
SHRI S. D. MUNDHRA	Geo-Miller & Co Pvt Ltd, Calcutta
SHRI U. C. MANKAD (*Alternate*)	
SHRI D. V. S. MURTHY	M. P. State Prevention & Control of Water Pollution Board, Bhopal
SHRI P. K. BANERJEE (*Alternate*)	
SHRI R. NATARAJAN	Bharat Heavy Electricals Ltd, Hyderabad
SHRI G. KRISHNAYYA (*Alternate* I)	
DR A. PRABHAKAR RAO (*Alternate* II)	
SHRI S. K. NEOGI	Institution of Public Health Engineers India, Calcutta
DR M. BANERJEE (*Alternate*)	
DR V. PACHAIYAPPAN	The Fertiliser Association of India, New Delhi
SHRI R. PARAMASIVAM	National Environmental Engineering Research Institute (CSIR), Nagpur
SHRI M. V. NANOTI (*Alternate*)	
SHRI A. K. PODDAR	Steel Authority of India Ltd, New Delhi
SHRI A. K. DAS (*Alternate*)	
SHRI H. S. PURI	Punjab State Board for the Prevention & Control of Water Pollution, Patiala
SHRI QIMAT RAI (*Alternate*)	

(*Continued on page* 2)

(*Continued from page* 1)

Members	*Representing*
SHRI B. B. RAO DR I. RADHAKRISHNAN (*Alternate*)	Ministry of Works & Housing
SHRI S. HANMANTH RAO SHRI C. H. GOVINDA RAO (*Alternate*)	Karnataka State Board for Prevention & Control of Water Pollution, Bangalore
SHRI G. S. RAY	The Fertilizer (Planning & Development) India Ltd, Sindri
DR S. K. ROY SHRI P. K. CHAKRAVARTY (*Alternate*)	The Alkali & Chemical Corporation of India Ltd, Calcutta
SHRI K. RUDRAPPA SHRI S. N. CHAKRABARTI (*Alternate*)	Engineers India Ltd, New Delhi
SHRI A. K. SAHA SHRI P. BHATTACHARYYA (*Alternate*)	National Test House, Calcutta
SHRI R. M. SHAH SHRI R. K. GANDHI (*Alternate*)	Tata Chemicals Ltd, Bombay
SHRI P. R. SHETH SHRI S. P. IYER (*Alternate*)	Excel Industries Ltd, Bombay
SHRI K. SURIYANARAYANAN SHRI G. C. GAUTAM (*Alternate*)	All India Distillers' Association, New Delhi
DR HARI BHAGWAN, Director (Chem)	Director General, ISI (*Ex-officio Member*)

Secretary

SHRI A. K. BAHL
Assistant Director (Chem), ISI

Panel for Methods of Test for Water and Effluents, CDC 26 : P1

Convener	
DR B. K. HANDA	Central Ground Water Board, New Delhi
Members	
SHRI Y. P. KAKAR (*Alternate* to Dr B. K. Handa)	
SHRI S. K. BHATTACHARJYA SHRI A. M. KULKARNI (*Alternate*)	Ion Exchange (India) Ltd, Bombay
DR S. H. CLERK	Industrial Toxicology Research Centre (CSIR), Lucknow
DR P. K. MATHUR	Bhabha Atomic Research Centre, Bombay
PROF R. S. MEHTA DR U. I. BHATT (*Alternate*)	Gujarat Water Pollution Control Board, Gandhinagar
DR D. V. S. MURTHY DR G. K. KHARE (*Alternate*)	M. P. State Prevention & Control of Water Pollution Board, Bhopal
DR S. P. PANDE SHRI M. V. NANOTI (*Alternate*)	National Environmental Engineering Research Institute (CSIR), Nagpur
DR N. M. PARHAD DR S. R. JOSHI (*Alternate*)	National Environmental Engineering Research Institute (CSIR), Nagpur
DR I. RADHAKRISHNANA SHRI M. R. PARTHASARTHY (*Alternate*)	Ministry of Works & Housing
SHRI G. S. RAY	The Fertilizer (Planning & Development) India Ltd, Sindri
REPRESENTATIVE	A. P. State Board for Prevention and Control of Water Pollution, Secunderabad
REPRESENTATIVE	Central Board for the Prevention & Control of Water Pollution, New Delhi
REPRESENTATIVE	Maharashtra Prevention of Water Pollution Board, Bombay
REPRESENTATIVE	National Institute of Oceanography (CSIR), Bombay
SHRI C. P. SHARDA SHRI M. S. DHINGRA (*Alternate*)	Shri Ram Institute for Industrial Research, Delhi
DR P. D. SHARMA SHRI D. C. MATHUR (*Alternate*)	Hindustan Copper Ltd, Khetri Copper Complex, Khetrinagar
DR (SMT) Z. R. TUREL SHRI K. C. MAHINDRA (*Alternate*)	The Institute of Science, Bombay

CONTENTS

AMENDMENT NO. 1 JANUARY 1985

TO

IS:1622-1981 METHODS OF SAMPLING AND MICRO-BIOLOGICAL EXAMINATION OF WATER

(First Revision)

(*Page 9, clause 3.2.4.3, line 2*) - Substitute '48' *for* '24' and add the following Note after this clause:

'NOTE—The incubation may be carried out for 72 hours where necessary'.

(CDC 26)

Printed at Simco Printing Press, Delhi

AMENDMENT NO. 2 DECEMBER 2000
TO
IS 1622 : 1981 METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

(*Page* 7, *clause* 3.1.1.1) — Insert the following new clause at the end of 3.1.1.1 and renumber the subsequent clauses:

'3.1.1.2 *Laminar flow air unit* — Vertical or horizontal kind for use of pouring sterile nutrient media into sterile petri dishes to avoid contamination.'

(*Page* 8, *clause* 3.1.1.5) — Insert the following at the end of first paragraph:

'If test tubes are used for culture work, these shall be plugged with non-absorbent cotton or caps and shall be sterilized and autoclaved at 121°C for 15 minutes.'

(*Page* 8, *clause* 3.2.1, *line* 11) — Substitute 'while higher plate counts give the earliest sign of contamination' *for* 'while rising ---------- pollution'.

(*Page* 9, *clause* 3.2.3.1, *line* 5) — Substitute '15 g of agar powder' *for* '1.5 percent agar powder'.

(*Page* 9, *clause* 3.2.4.1, *line* 4) — Insert the word 'sterile' before the words 'petri dish'.

(*Page* 9, *clause* 3.2.4.4, *last senntence*) — Substitute 'Record the number of incubation and days and temperature of incubation' *for* 'Record the number of ---------- incubations'.

(*Page* 9, *clause* 3.3.1, *line* 19) — Insert 'Most Probable Number' before the word 'MPN'.

[*Page* 10, *clause* 3.3.1.1(b), *line* 22] — Substitute 'sterlize at 115°C for about 15 minutes (not exceeding 30 minutes) in the autoclave at 1.02 + 0.03 kg/cm^2 (15 + 0.5 psi) gauge pressure' *for* 'sterlize at 115°C for 10 minutes in the autoclave'.

(*Page* 11, *clause* 3.3.1.1) — Insert the following note after 3.3.1(h)(iv):

'NOTE — Lugol's iodine is used as mordant and ethyl alcohol is used as a decolourizer.'

Amend No. 2 to IS 1622 : 1981

[*Page* 11, *clause* 3.3.1.2(c)(iii)] — Substitute 'Gram-stain' *for* 'gram — stain'.

[*Page* 11, *clause* 3.3.1.2(c)(iv)] — Substitute 'from the agar slant' *for* 'on the agar slant'.

[*Page* 13, *clause* 3.3.5.2(iii), *line* 3] — Substitute 'cyclohexamide' *for* 'cyclohexamine'.

[*Page* 15, *clause* 3.4.1.2(b), *line* 12] — Substitute 'MPN' *for* 'MP '.

(*Page* 15, *clause* 3.4.2.2 , *line* 13) — Substitute '10 g of agar' *for* '1 percent agar'.

(*Page* 16, *clause* 3.5.4.3 , *line* 7) — Substitute 'Wash, dry and examine under oil immersion, using a compound microscope' *for* 'Wash, dry, mount and observe under microscope'.

(*Page* 17, *clause* 3.8.2.1, *line* 10) — Substitute '0.03 kg/cm^2' *for* '0.3 kg/cm^2'.

(*Page* 17, *clause* 3.8.2.1, *line* 12) — Substitute 'about 15 minutes but not exceeding 30 minutes' *for* '10 minutes'.

(CHD 12)

Printed at Simco Printing Press, Delhi

AMENDMENT NO. 3 DECEMBER 2002
TO
IS 1622 : 1981 METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

[*Page* 13, *clause* **3.3.5.3**(iii), *line* 3] — Substitute 'cyclohexamide' *for* 'cyclohexamine'.

(*Amendment No.* 2, *page* 2, *lines* 5 *and* 6 *related to clause* **3.3.5.2**) — Delete.

(CHD 32)

Printed at Simco Printing Press, Delhi

AMENDMENT NO. 4 MAY 2003
TO
IS 1622 : 1981 METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

(*Page* 17, *clause* 3.7.4) —Substitute the following for the existing:

'3.7.4 *Detection of Sulphate Reducing Bacteria*

Distribute 10 ml of double strength medium (double the quantity of medium constituents in 1 000 ml distilled water) in 5 test tubes. Similarly, distribute 5 ml of single strength medium in 10 test tubes. Sterilize at 1.02 ± 0.03 kg/cm^2 gauge pressure (15±0.5 psi gauge pressure and 121°C temperature approximately) for 15 min.

Add the following samples aseptically as stated below and mix well:

Add 10 ml of the sample to 5 double strength sterile test tubes. Add 1 ml of the sample to 5 single strength sterile test tubes. Add 0.1 ml of the sample to 5 single strength sterile test tubes. Cover the surface of each test tube by sterile liquid paraffin to a depth of 1-2 mm. Incubate the tubes at 28-30°C for 5-7 days.

The production of black colour in medium will indicate the presence of Sulphate Reducing Bacteria (SRB).

After incubation, observe and note down the tubes showing black colour. Compare the tubes showing black colour from double strength and single strength media. With the help of McCrady's chart, quantify the number of SRB in the water sample.

NOTE —If soil sample is to be analysed, then take 1 000 mg, 100 mg, 10 mg in five test tubes each. Add 10 ml of the double strength medium in the 1 000 mg tubes and 5 ml of the single strength medium in each of the rest 10 test tubes. Follow the rest of the procedure as described above."

(*Page* 25, *Table* 3) — Insert the following Table 4 after Table 3:

Table 4 Most Probable Number (MPN) of Organisms Present per 100 ml of Sample and Confidence Limits using 5 Tubes of 10 ml , 5 Tubes of 1 ml and 5 Tubes of 0.1 ml

(*clause* 3.3.1.3)

Combination of Positives	MPN Index/ 100 ml	95 percent Confidence Limits		Combination of Positives	MPN Index/ 100 ml	95 percent Confidence Limits	
		Lower	Upper			Lower	Upper
				4-2-0	22	9.0	56
0-0-0	< 2	—	—	4-2-1	26	12	65
0-0-1	2	1.0	10	4-3-0	27	12	67
0-1-0	2	1.0	10	4-3-1	33	15	77
0-2-0	2	1.0	13	4-4-0	34	16	80
				5-0-0	23	9.0	86
1-0-0	2	1.0	11	5-0-1	30	10	110
1-0-1	4	1.0	15	5-0-2	40	20	140
1-1-0	4	1.0	15	5-1-0	30	10	120
1-1-1	6	2.0	18	5-1-1	50	20	150
1-2-0	6	2.0	18	5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
				5-5-0	240	100	940
4-0-0	13	5.0	38	5-5-1	300	100	1 300
4-0-1	17	7.0	45	5-5-2	500	200	2 000
4-1-0	17	7.0	46	5-5-3	900	300	2 900
4-1-1	21	9.0	55	5-5-4	1 600	600	5 300
4-1-2	26	12	63	5-5-5	1 600	—	—

(CHD 32)

Reprography Unit, BIS, New Delhi, India

representing the container and shall be transferred immediately to thoroughly clean and dry containers, sealed air-tight and labelled with particulars given in **A-1.5**. The individual samples so obtained shall be divided into three sets in such a way that each set has a sample representing each selected container. One of these sets shall be marked for the purchaser, the other for the vendor, and the third for the referee.

A-3.2 Preparation of a Composite Sample — From the material from each selected container remaining after the individual sample has been taken, equal quantities of the material shall be taken and mixed together so as to form a composite sample weighing not less than 360 g. This composite sample shall be divided into three approximately equal parts and transferred to clean and dry glass containers and labelled with the particulars given in **A-1.5**. One of these composite samples shall be marked for the purchaser, the other for the vendor, and the third for the referee.

A-3.3 Referee Samples — Referee samples shall consist of a set of individual samples (*see* **A-3.1**) and a composite sample (*see* **A-3.2**) marked for this purpose and shall bear the seals of the purchaser and the vendor. These shall be kept at a place agreed between the two.

A-4. NUMBER OF TESTS

A-4.1 Tests for requirements in respect of barley-grain content and protein shall be conducted on each of the samples constituting a set of individual test samples (*see* **A-3.1**).

A-4.2 Tests for the remaining characteristics, namely, moisture, total ash, acid insoluble ash, crude fibre and alcoholic acidity, shall be conducted on the composite sample (*see* **A-3.2**).

A-5. CRITERIA FOR CONFORMITY

A-5.1 The lot shall be considered satisfactory in respect of the requirements of **A-4.1**, if each individual sample satisfies all the requirements.

A-5.2 The lot shall be considered satisfactory in respect of the requirements of **A-4.2**, if the test results on the composite sample satisfy the corresponding requirements.

A-5.3 The lot shall be declared to be in conformity with all the requirements of this specification, if it has been found satisfactory in accordance with **A-5.2**.'

(*Page* 4, *Clause* **C-1.1**) — Substitute the following for the existing clause:

'**C-1.1 Preparation of Sample** — Grind in a pestle and mortar about 100 g of the material so that at least 90 percent passes through

425-micron IS Sieve. Transfer this prepared sample to a well stoppered glass bottle for use as indicated under **C-1.2, F-3.1, G-2.1** and **H-2.1.**

NOTE — In case 425-micron IS Sieve (conforming to IS : 460-1962) is not available, BS Test Sieve 36, ASTM Sieve 40 or Tyler Test Sieve 35, which have their apertures within the limits specified for this IS Sieve, may be used.'

(*Page* 7, *Clause* **H-1.2**) — Substitute the following for the existing clause:

'**H-1.2 Standard Sodium Hydroxide Solution** — 0·05 N.'

(*Page* 7, *Clause* **H-1.3**) — Substitute the following for the existing clause:

'**H-1.3 Phenolphthalein Indicator Solution** — Dissolve 0·1 g of phenolphthalein in 100 ml of 60 percent rectified spirit.'

(*Page* 7, *Clause* **H-2.1**) — Substitute the following for the existing clause:

'**H-2.1** Weigh 5 g of the sample into a conical glass stoppered flask and add 50 ml of 90 percent alcohol (by volume) previously neutralized against phenolphthalein. Stopper, shake and allow to stand for 24 hours, with occasional shaking. Filter the alcoholic extract through a dry filter paper. Titrate 10 ml of the combined alcoholic extract against the standard sodium hydroxide using phenolphthalein as indicator. Calculate the percentage of alcoholic acidity as sulphuric acid.'

(*Clause* **H-3.1**, *line* 3) — Substitute '$\frac{24{\cdot}52\ AN}{W}$' *for* '$\frac{12{\cdot}25\ AN}{W}$'.

Indian Standard

METHODS OF SAMPLING AND MICROBIOLOGICAL EXAMINATION OF WATER

(First Revision)

0. FOREWORD

0.2 This Indian Standard (First Revision) was adopted by the Indian Standards Institution on 30 November 1981, after the draft finalized by the Water Sectional Committee had been approved by the Chemical Division Council.

0.2 Microbiological examination of water includes both bacteriological and biological examinations. Bacteriological examination of water is necessary for determining its fitness for use for human consumption, and for use in industries such as food processing and dairy, photofilm, etc. Water used for drinking, food processing and dairying should be free from faecal or sewage contamination because microorganisms causing water-borne diseases such as typhoid and paratyphoid fevers, food poisoning, gastroenteritis, cholera, dysentery and diarrhoea are excreted in the faeces of individuals suffering from the disease. The detection of these pathogenic organisms in a sample of water is difficult and may not always be accomplished with certainty. Bacterial organisms of the coliform and faecal streptococci groups, however, inhabit the intestinal tract of man and animals in great abundance and are readily detectable. Hence their presence in a sample of water is looked upon as an indication of the probable presence of intestinal pathogenic organisms, while their absence from water usually precludes the presence of such pathogens. Tests for the presence of *Clostridium welchii* are also carried out on samples of water for obtaining supplementary evidence of faecal pollution.

0.2.1 The presence of organisms belonging to the groups such as iron bacteria, sulphur bacteria, sulphate reducing bacteria, slime-forming bacterial and gelatin liquefying bacteria is undesirable in water used for drinking purposes, air-conditioning, paper manufacture and many other industrial uses. Some of these organisms are known to cause corrosion.

0.2.2 Algae and other microscopic plants and animal-life in water may cause odour and taste problems and also affect suitability of the water for use in various industries. However, this standard includes only microscopic examination and enumeration of these organisms.

0.3 This standard was first published in 1964. This revision incorporates the two amendments issued to IS : 1622-1964*, the membrane filter technique for coliforms and faecal streptococci, test for faecal coliforms, delayed incubation method for total coliforms and spore staining technique for *clostridium welchii*.

0.4 This standard prescribes laboratory preparation of culture media. However, dehydrated media commercially available may also be employed. Since preparation of culture media and solutions is a critical aspect of water quality testing, the date of receipt of media (dehydrated powder medium) and the date of opening should be recorded. Where practical, 125 g bottles should be purchased to ensure minimum exposure.

0.4.1 When a new lot of media is used, the contents should be tested for expected performance. It should be stored in a cool, dry place away from sunlight.

0.5 It is recommended that laboratory pure water suitable for microbiological applications should be used as far as possible. For the purpose reference may be made to Standard Methods for the Examination of Water and Wastewater. 1975 Ed 14. (Geldreich, E. E. and Clark H. F. 1965 Distilled water suitability for microbiological applications. *J. Milk Food Technol* 28 : 351)

0.6 In the preparation of this standard, considerable assistance has been derived from the following publications, and the draft was prepared by National Environmental Engineering Research Institute, Nagpur:

International standards for drinking water. 1971. World Health Organization, Geneva.

Environmental microbiology — A course outline, NEERI, Nagpur.

*Methods of sampling and test for microbiological examination water used in industry.

Bacteriological Examination of Water supplies. Report No. 71, 1969. Her Majesty's Stationery Office, London.

Standard methods for the examination of water and wastewater. 1975 Ed 14. American Public Health Association; American Water Works Association; and Water Pollution Control Federation U.S.A.

Biological methods for monitoring the environment, EPA — 1978.

0.7 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*.

1. SCOPE

1.1 This standard prescribes methods of sampling and microbiological examination of water.

2. SAMPLING

2.1 Sampling for Bacteriological Examination

2.1.1 *Sampling Bottles* — Samples for bacteriological examination shall be collected in clean, sterilized, narrow mouthed neutral glass bottles of 250, 500, or 1 000 ml capacity. The bottle shall have a ground glass stopper having an overlapping rim. The stopper shall be relaxed by an intervening strip of paper between the stopper and the neck of the bottle. The stopper and the neck of the bottle shall be protected by paper or parchment cover. The bottle shall be sterilized in hot air oven at 160°C for one hour or an autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. The sampling bottle shall not be opened except at the time of sampling.

NOTE 1 — Discard bottles which have chips, cracks and etched surfaces. Before use, bottles should be thoroughly cleaned with detergent and hot water, followed by a hot water rinse to remove all traces of detergents. Then rinse them three times with laboratory pure water.

NOTE 2 — A chelating agent should be added to sample bottles used to collect samples, suspected to contain more than 0·01 mg/l of heavy metals such as copper, lead, zinc, nickel, etc. Add 0·3 ml of 15 percent EDTA — Na_4 salt for each 125 ml of sample.

2.1.1.1 *Dechlorination* — If the water to be sampled contains or is likely to contain chlorine, sodium thiosulphate shall be added to the clean, dry sampling bottles before sterilization in an amount to provide an approximate concentration of 100 mg/l in the sample. This can be done by adding 0·5 ml of 5 percent thiosulphate solution to a 250-ml bottle. Sterilize in an autoclave.

2.1.2 *Sampling Procedure* — The samples shall be representative of the water to be tested and they should be collected with utmost care to ensure that no contamination occurs at the time of collection or prior to examination. The sample bottle shall not be opened till the time of filling. The stopper shall be removed with care to eliminate soiling. During sampling, the stopper and the neck of the bottle shall not be touches and they shall be protected from contamination. The bottle shall be held near the base, filled without rinsing, and the stopper replaced immediately. Then the brown paper wrapping should be tied to protect the samples from contamination.

2.1.2.1 *Sampling from taps* — Flame the tap (in case of plastic tap, apply alcohol or spirit, preferably rectified, and allow it to dry). The tap shall be opened fully and the water allowed to run to waste for two to three minutes or for a sufficient time to permit clearing of the service line. The flow from the tap shall then be restricted to permit filling the bottle without splashing. Leaking taps, which allow water to flow over the outside of the tap should be avoided as sampling points.

NOTE — If the tap is connected to an overhead storage tank, this fact should be recorded in the sampling report.

2.1.2.2 *Sampling direct from a source* — When the sample is to be collected directly from a stream, river, lake, reservoir, spring, or a shallow well, it shall be representative of the water that will be taken for supply to the consumers. Hence a sample shall not be taken too far from a point of draw-off or too close. Areas of relative stagnation in a stream should be avoided. Samples from a river, stream, lake, or a reservoir can often be taken by holding the bottle in the hand near its base and plunging it neck downward, below the surface. The bottle shall then be turned until the neck points slightly upward, the mouth being directed against the current. If no current exists, as in a reservoir, a current shall be artificially created by pushing the bottle horizontally forward in a direction away from the hand. If it is not possible to collect samples in this way, a weight may be attached to the base of the bottle which can

*Rules for rounding off numerical values (*revised*).

then be lowered into the water. In any case, damage to the bank should be guarded against, otherwise fouling of the water can occur. Special apparatus which permits mechanical removal of the stopper of the bottle below the surface is required to collect samples from the depths of a lake or a reservoir. If the sample is to be taken from a well, fitted with a hand-pump, flame the mouth of the hand-pump (in case of a plastic mouth, apply alcohol or spirit, preferably rectified, and allow it to dry) and pump the water to waste for four to five minutes before the sample is collected. If the well is fitted with a mechanical pump, the sample should be collected from a tap on the discharge. (If the tap is connected to an overhead storage tank, this fact should be recorded in the sampling report.) If there is no pumping machinery, the sample can be collected directly from the well in a sterilized bottle fitted with a weight at the base. In this case, care shall be taken to avoid contamination of the sample by any surface scum. Where it is not possible to collect the sample directly into the bottle, as for example where there is a high bank the sample may be obtained by means of suitable metal jug. The jug is sterilized by pouring into it a teaspoonful of methylated spirit and tilting the jug in such a way that the spirit comes in contact with the entire inner surface of the jug, and igniting. The jug shall be lowered to the required depth and then drawn up and down two or three times before it is brought to the surface. It shall be rinsed out at least twice before the sample is taken. Should the jug come in contact with the bottom or skid along the surface so that it may have collected the surface film, the sample shall be discarded, the jug re-sterilized and another sample drawn. The water from the jug shall be poured into the bottle and the glass stopper of the bottle be replaced, care being taken to avoid the cover being caught between the stopper and the neck of the bottle.

2.1.3 *Size of the Sample* — The volume of the sample shall be sufficient for carrying out all the tests required. The sampling bottle should not be filled up to the brim and 2 to 3 cm space should be left for effective shaking of the bottle.

2.1.4 *Preservation and Storage* — The initial time limit for starting analysis should be 1 hour but not more than 6 hours after collection of water samples. Under exceptional circumstances the analysis should be commenced at least within 30 hours and sample should be kept in dark at 1-4°C. If sampling and transit time requires more than 6 hours, temporary field laboratory should be set up or the delayed incubation procedure (*see* **3.3.5**) should be adopted if MF technique is used.

2.1.5 *Identifying Data* — All samples shall be legibly marked with the source of the sample, date and time of collection, and the name and designation of the person collecting the sample. As results of laboratory examination of the sample shall always be considered in conjunction with the sanitary survey of the water supply system, it is important that when submitting a sample for analysis, complete and accurate data of the nature and source of the supply, topography of the water shed, possibility of pollution gaining access to the source, methods of treatment adopted, the condition of the distribution system, and such other information as would be relevant from sanitary viewpoint is furnished. It shall be ascertained whether the tap from where the sample is collected is supplying water from a service pipe directly connected with the main or with a cistern or a storage tank. Specimen form for such information is given in Appendix A.

2.2 Sampling for Biological Examination

2.2.1 *General Considerations* — The microscopic organisms other than bacteria in water include a large variety of algae, moulds or fungi, yeasts, protozoa, rotifers, crustacea, animalcula, etc many of which affect the quality of water for drinking and industrial uses. Those organisms which occur free-floating in water are collectively known as plankton, while those which inhabit the bottoms of the tanks and streams or are attached to the stones or other submerged objects are called periphyton. The examination of the nature and number of the organisms present in the sample is of use in understanding the nature of pollution, the cause of undesirable tastes and odours, slime growth, ecosystem imbalances, etc.

NOTE — The sampling report should mention the time, depth and frequency of sampling.

2.2.2 *Procedure for Sampling*

2.2.2.1 The sample of natural plankton-producing water shall be collected in clean, neutral glass bottles of 2-litre capacity, fitted with ground glass stopper. The bottle shall not be filled completely and a small air space shall be left below the stopper.

2.2.2.2 A concentrated sample or catch of the plankton organisms and other particulate suspended matter in the water shall be collected with the aid of a plankton net. The net shall be conical in shape, of suitable size, with a circular mouth and made of bolting silk cloth with more than 6 000 meshes per square centimetre. The net shall be hauled through the water in an oblique or horizontal direction for a certain distance, lifted from the water, allowed to drain and the organisms in the net washed down into a container by splashing water on the outer surface of the net. The

catch shall then be made up to a known volume with the water. Nets provided with closing devices shall be used for collecting samples of plankton from different depths.

2.2.2.3 For quantification of the plankton it is recommended to strain known volume of water through plankton net. Number of organisms caught in the catch may be used to calculate back the original number per unit volume of the sample.

2.2.2.4 Sampling phytoplankton with nets provides data of limited value since the total count, volume, bio-mass and species composition are not measurable. Because of selectivity of mesh size the smaller plankton (nanoplanktons) which may contribute as much as 60 percent of the total bio-mass are not collected. In such situation sedimentation membrane filtration or centrifugation are recommended.

2.2.3 The samples shall be examined within 2 to 3 hours after collection, when the organisms are alive. If this is not possible, the samples shall be preserved in ice or in the refrigerator (3 to 4°C) for a few days taking care not to allow it to freeze. If the examination is to be made later, the samples shall be preserved as follows:

To each 100 ml of the sample, add about 3 ml of 2 percent formaldehyde solution (made by diluting 5 ml of 37 — 40 percent aqueous formaldehyde solution to 100 ml with distilled water), 0·5 ml of 20 percent detergent solution made by diluting 20 ml of liquid detergent to 100 ml with distilled water and 5 — 6 drops of copper sulphate solution 21 percent (*v/v*). This preservative maintains cell colouration and is effective indefinitely. Store the preserved sample in the dark.

3. BACTERIOLOGICAL EXAMINATION

3.1 General Equipment

3.1.1 *Equipment*

3.1.1.1 *General* — It is essential for accurate and satisfactory laboratory work that good equipment in proper working order be provided. Thus, the minimum laboratory equipment listed, must be available in an approved laboratory and all items should meet the minimum requirements given. Additional items of equipment not listed, will be required in an approved laboratory and they should meet similar standards of quality and operation.

3.1.1.2 *Incubators* — Incubators should maintain a uniform and constant temperature (35-37°C or 44-45°C) at all times in all parts. This can be accomplished by the use of a water-jacketed or anhydric type of incubator, with thermostatically controlled low-temperature electric heating units properly insulated and located in or adjacent to walls or floor of chamber, and preferably equipped with mechanical means of circulating air.

Incubators should also be provided with shelves spaced to ensure uniformity of temperature throughout the chamber. The inside dimensions of the chamber should be at least 50 × 50 cm at the base and 60 cm high, to accommodate a maximum of 200 Petri dishes; 2·5 cm space should be provided between adjacent stacks of plates and between walls and stacks.

Accurate thermometers, with bulb continuously immersed in liquid (glycerine, water or mineral oil), should be maintained within the incubator and daily readings of the temperatures should be recorded. In addition, it is desirable to maintain a maximum and minimum registering thermometer within the incubator on the middle shelf to record temperature variations over a 24-hour period. Temperature variations within the incubator filled to maximum capacity should be determined at intervals. It is recommended that a recording thermometer be installed in every incubator whenever possible, so that a permanent record of temperature variations within the incubating chamber may be kept.

Incubators equipped with high-temperature heating units are unsatisfactory, since such sources of heat frequently cause localized overheating. Incubators, so heated may be made to operate satisfactorily by replacing the high temperature units by suitable wiring, arranged to operate at a lower temperature, and by installing mechanical air circulation. It is desirable, where ordinary room temperatures very excessively, that laboratory incubators be kept in special rooms which may be maintained at a few degrees below the recommended incubator temperature.

3.1.1.3 *Water-baths* — Water-baths are useful for carrying out the 44°C fermentation test. They should be capable of maintaining a temperature of 44°C-45°C. They should be equipped with mercury-toluol or other reliable thermostats for sensitive regulation of the temperature, and should be adequately insulated against heat loss. An accurate thermometer should be provided, with its bulb placed at the level of the medium in the fermentation tubes. A continuous-recording thermometer is advisable.

3.1.1.4 *Sterilizers*

a) *Ovens* — Hot-air sterilizing ovens should be of sufficient size to prevent crowding of the interior and constructed to give uniform and adequate sterilizing temperatures, and equipped with suitable thermometers capable of registering

accurately in the range 160-180°C. The use of temperature-recording instrument is optional.

b) *Autoclaves* — Autoclaves should be of size sufficient to prevent crowding of the interior, and constructed to provide uniform temperatures within chambers up to and including the sterilizing conditions of 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately). They should be equipped with pressure gauges, properly adjusted safety valves, and accurate thermometers with bulb properly located on exhaust line, so as to register minimum temperature within sterilizing chambers (temperature recording instrument optional). In emergencies, a pressure-cooker may be substituted for an autoclave, if results have previously been demonstrated to be satisfactory with this method.

3.1.1.5 *Glassware* — Clean glassware is critical to ensure valid results. Previously used or new glassware should be thoroughly cleaned with phosphorus-free laboratory detergent and hot water, followed by at least three rinses with laboratory pure water. Generally, pipettes, dilution bottles and petri dishes are required.

a) *Pipettes* — Pipettes may be of any convenient size (generally 1 ml or 10 ml) provided it is found by actual test that they deliver accurately the required amount in the manner in which they are used. The error of calibration should not exceed 2·5 percent. Pipettes with unbroken tips and with graduations distinctively marked should be used. Pipettes with damaged tips should be repaired or discarded.

b) *Dilution bottles* — Bottles or tubes of resistant glass, preferably Pyrex, closed with glass stoppers, rubber stoppers, or screw caps equipped with liners that do not produce toxic or bacteriostatic compounds on sterilization, should be used. Cotton plugs shall not be used as closures. Graduation levels should be indelibly marked on the side.

c) *Petri dishes* — Petri dishes 100 mm in diameter, with the side wall at least 15 mm high, should be used with glass or porous tops, as preferred. The bottoms of the dishes should be free from bubbles and scratches and should be flat, so that the medium will be of uniform thickness throughout the plate. When available, sterilized disposable plastic petri dishes may be used as an alternative.

3.1.1.6 *Inoculating needle and loop* — It shall be 4 mm in diameter formed at one end of a length of 0·375 mm thick wire of nichrome, platinum or platinum-iridium alloy. It shall be 38 mm long from the loop to the holder. The holder consists of a thin metal rod or tube. It shall be sterilized by flaming.

3.1.1.7 *Refrigerators* — An approved laboratory should have a refrigerator of sufficient capacity for the required work load and capable of maintaining a continuous temperature between 0°C and 5°C. An electrically operated refrigeration unit provides the most efficient service.

3.1.1.8 *Colony counter* — An effective device for examining colonies, providing a magnification of 3×, should be available. In general, a Quebec or similar colony counter will be suitable for this purpose.

3.2 Standard Plate Count

3.2.1 *General* — Standard plate count (which is an empirical method) serves to indicate the efficiency of certain processes in water treatment, particularly coagulation, filtration and disinfection and the cleanliness of the mains, reservoirs, etc. It provides an estimate of the general hygienic quality of water, which is important where large scale preparation of food and drink is concerned. Low counts are of importance for avoiding food spoilage, while rising plate counts give the earliest sign of pollution.

3.2.1.1 The standard plate count method is a direct measurement of the viable aerobic and facultative anerobic bacteria in a water environment capable of growth on the selected plating medium. The procedure does not allow the more fastideous aerobes or obligate anaerobes to develop. Also the bacteria of possible importance in water such as Crino-thrix, sphaerotilus and actinomycetes will not develop within the incubation period specified for potable water. Clumps of organisms in the water sample which are not broken up by shaking result in under estimate of bacterial density. Since an aggregate of cells will appear as one colony on the growth medium. The number of types of bacteria that develop are influenced by the time and comparative temperature of incubation, the *p*H of the medium, the level of the oxygen, the presence of specific nutrients on the growth medium competition among cells for nutrients, antibiosis, medation, etc.

3.2.2 In solid medium counting of organisms depends on the fact that living cells will proceed to multiply and in time will produce sufficient progeny to form a colony visible to naked eye. Since bacteria occur in water as single cells, pairs, groups, chains or even dense clumps, not every individual living cell will

develop into a separate colony on incubation. Therefore, number of colonies appearing on a plate does not necessarily represent the total number of organisms present in test volume. The results are expressed as number of colonies per ml.

3.2.3 *Medium and Reagent*

3.2.3.1 *Nutrient agar* — Dissolve 1 g glucose, 5·0 g of peptone and 3·0 g of beef extract in 1 000 ml of distilled water. Adjust the *p*H to 7·2, distribute in required quantity and add 1·5 percent agar powder. Sterilize at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes in the autoclave.

3.2.3.2 *Dilution water*

a) *Buffered dilution water* — To prepare stock phosphate buffer solution, dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust *p*H to 7·2 with sodium hydroxide solution (1 N) and dilute to 1 litre with distilled water.

   Add 1·25 ml of stock phosphate buffer solution to 1 litre of distilled water. Dispense in amounts that will provide 18 ± 0·4 ml or 9 ± 0·2 ml in 150 × 25 mm or 150 × 18 mm test tubes respectively. Sterilize in autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes.

b) *Quarter strength ringer's solution* — Dissolve 9·00 g of sodium chloride, 0·42 g of potassium chloride, 0·48 g of calcium chloride and 0·20 g of sodium bicarbonate in 1 litre of water. This solution is known as Ringer's solution. Dilute 500 ml of this solution to 2 litres to obtain quarter strength Ringer's solution. Dispense in amounts that will provide 18 ± 0·4 ml or 9 ± 0·2 ml in 150 × 25 mm or 150 × 18 mm test tubes respectively. Sterilize in autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes.

3.2.4 *Procedure*

3.2.4.1 *Preparation and dilution* — Shake the samples about 25 times. Withdraw required portion with a sterile pipette, and introduce into the petri dish or dilution tube.

3.2.4.2 *Plating* — Place 1·0 ml, or 1·0 ml of other suitable dilution to be used for plating in the petri dish first. Then add to the petri dish 10 to 15 ml of melted nutrient agar medium at a temperature of 43 to 45°C (tolerable to the skin). The nutrient agar and the sample shall be thoroughly mixed over the bottom of the petri dish by tilting and rotating the dish several times. Allow the plate to solidify and place immediately in the incubator in an inverted position.

3.2.4.3 *Incubation* — Incubate the plates at 37°C for 24 hours.

3.2.4.4 *Counting* — In preparing plates, plant such amounts of water for dilution which will give from 30 to 300 colonies on a plate. Always have two or more plates for each dilution. Report the result as the average of all plates falling within limits. It is not desirable to plant more than 1·0 ml in a plate. If the colonies are more than 300 or less than 30 from 1 ml sample, disregard it. In practice, counts less than 30 occur when chlorinated water samples are plated. When the number of colonies is more than 300 in a plate, report the count at ' TNC ' (too numerous to count). Counting shall be done with an approved counting aid, such as colony counter. Record the number of colonies to the nearest 5 units per ml and report the temperature of incubations.

3.3 Test for Coliforms

3.3.0 The coliform group includes all of the aerobic and facultative anaerobic gram negative, non-spore forming rod shaped bacteria which ferment lactose with gas formation within 48 hours at 37°C. The standard test for the estimation of number of the coliform groups may be carried out either by the multiple tube dilution test (presumptive test, confirmed test, or completed test) or by the membrane filter technique.

3.3.1 *Multiple Tube Dilution Test (MTD)* — The presumptive, confirmed and completed tests are presented as total independent procedures. In using these procedures, the worker must know what is to be the stage at which the test is to be ended, and details of the procedure throughout. Thus, if the worker knows that the test will be ended at the confirmed test, he will stop at the confirmed test stage only. All the necessary information regarding the sample should be recorded. It is convenient to express the results of the examination of replicate tubes and dilutions in terms of most probable number. This term is actually an estimate based on certain probability formulae. The most satisfactory information is obtained when the largest portion examined shows no gas in all or a majority of the tubes. The MPN value for a given sample is obtained by the use of MPN tables. Standard practice in water analysis is to plant five tubes for each dilution and a minimum three different dilutions are employed. The results are to be

recorded in the proper form. Table of MPN are given in Appendix B.

3.3.1.1 *Media and reagents*

a) *Dilution water — see* **3.2.3.2.**

b) *MacConkey broth* — This is used as a presumptive medium for the enumeration of coliform bacteria in water samples. Its composition is an as under:

Peptone	20 g
Lactose	10 g
Sodium chloride	5 g
Bile salt	5 g
Distilled water	1 000 ml

In place of bile salt, which is a commercial product, sodium taurocholate or sodium tauroglycocholate may be used.

Dissolve all the ingredients and adjust the *p*H to 7·4. After adjusting the *p*H, add 1 ml of 1 percent alcoholic solution of bromocresol purple or 5 ml of 1 percent aqueous solution of neutral red. This will be the single strength medium. Distribute 10 ml of the medium into 150 × 15 mm test tubes and add a Durham's tube (25 × 5 mm) in an inverted position. Plug the tubes with non-absorbent cotton and sterilize at 115°C for 10 minutes in the autoclave. This medium is used for 1 ml and the decimal dilutions of the water sample. For 10 ml and larger aliquots a double strength medium is used. For the double strength medium add the above ingredients in double the quantities in 1 000 ml of distilled water. This medium is dispensed into 10 ml quantities in 150 × 18 mm test tubes added with Durham's tube and sterilized.

c) *Brilliant Green bile lactose broth (BGB)* — This medium is used as a confirmatory test for coliforms as well as for faecal coliforms. Its composition is as under:

Peptone	10 g
Lactose	10 g
Bile salt	20 g
Distilled water	1 000 ml

Dissolve all the ingredients and adjust the *p*H to 7·4. Add 1 33 ml of 1 percent aqueous solution of brilliant green indicator. Distribute 4 ml quantities into 150 × 12 mm test tubes and add a Durham's tube to each. After plugging with non-absorbent cotton, sterilize at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes in the autoclave.

d) *Peptone water* — This is used for indole test or for preparing a liquid culture of an organism. Its composition is as follows:

Peptone	10 g
Sodium chloride	5 g
Distilled water	1 000 ml

Dissolve all the ingredients. Adjust the *p*H to 7·4. Dispense 4 ml medium into 100 × 12 mm tubes and plug with non-absorbent cotton. Sterilize in the autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes.

e) *Mac Conkey agar* — The medium is used for the completed test or for IMViC classification of coliforms. Its composition is as under:

Peptone	20 g
Lectose	10 g
Sodium chloride	5 g
Bile salt	5 g
Distilled water	1 000 ml

Dissolve all the ingredients and adjust the *p*H to 7·4. Add 10 ml of 1 percent aqueous solution of neutral red indicator and 15 g of agar. Steam the medium for 15 to 30 minutes so that agar is dissolved properly and sterilize in autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. After sterilization, cool to 45°C and prepare the plates by pouring 15 ml of melted agar per plate.

Allow to solidify, invert and incubate at 37°C for drying as well as for sterility test.

f) *Nutrient agar slants* — Prepare the nutrient agar as prescribed in **3.2.3.1.** Dispense while in the melted condition about 10 ml quantity into each tube (150 mm × 15 mm). Sterilize in the autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. After sterilization the slants are prepared by keeping the tubes in a slanting position and allow them to solidify. Unless they are to be used, they should be stored in a refrigerator.

g) *Kovac's reagent* — It is used for indole test. Its composition is as under:

Paradimethyl aminobenzaldehyde	5 g
Amyl alcohol or n-butanol	75 ml
Concentrated hydrochloric acid	25 ml

Dissolve paradimethyl aminobenzaldehyde in amyl alcohol and then add 25 ml of hydrochloric acid. The reagent shall be yellowish in colour. Store in amber coloured glass stoppered bottle.

h) *Gram staining reagents*

i) Crystal violet is used as a primary stain.

Solution A —	Crystal violet (85 percent dye content)	2 g
	Ethyl alcohol (95 percent)	20 ml
Solution B —	Ammonium oxalate	0·8 g
	water	80 ml

Mix solutions A and B in equal parts. It is sometimes found, however, that this gives so concentrated a stain that gram-negative organisms do not properly decolorize. To avoid this, dilute solution A as much as ten times. Use 20 ml of this diluted solution and mix with solution B.

ii) *Lugol's iodine* — Dissolve 1 g of iodine crystals and 2 g of potassium iodide in 300 ml of distilled water.

iii) Safranin is used as a counter stain. Dissolve 25 g of safranin dye in 100 ml of 95 percent ethyl alcohol. Add 10 ml of the solution to 100 ml of distilled water.

iv) Ethyl alcohol — 95 percent.

3.3.1.2 *Procedure* — Shake the water samples thoroughly before making dilutions or before inoculation.

a) *Presumptive test*:

i) Use MacConkey broth. Inoculate a series of fermentation tubes with appropriate measured quantities of the water to be tested. The concentration of nutritive ingredients in the mixture should be sufficient and according to requirements. Ten ml and above aliquots should be inoculated in double strength and 1 ml and its dilution should be inoculated into single strength medium.

ii) Incubate all tubes at 37°C for 24 to 48 hours. Examine each tube at the end of 24 ± 2 hours for gas production and if no gas has been formed, reincubate for another 24 hours and at the end of 48 hours, examine again. Record the presence of or absence of gas at each examination of the tubes regardless of the amount.

iii) Formation of the gas within 48 ± 3 hours in any amount, in the inner fermentation tubes, constitutes a possible presumptive test. The absence of gas formation at the end of 48 ± 3 hours of incubation constitutes a negative test.

b) *Confirmed test* — The medium used for confirmed test is brilliant green bile lactose broth (BGB).

i) Submit all primary fermentation tubes showing any amount of gas at the end of 24 hours incubation to the confirmed test. If additional primary fermentation tubes show gas at the end of 48 hours incubation, these too shall be submitted to the confirmed test. Use a sterile metal loop, 3 to 4 mm in diameter to transfer one or two loopful of medium from the presumptive positive tubes to a tube of BGB broth. When making such transfers, gently shake the tube first or mix by rotating. Incubate the inoculated tubes at 37°C for 48 ± 3 hours.

ii) The formation of gas in any amount in the Durham's tubes of BGB tube at any time within 48 ± 3 hours constitutes a positive confirmed test.

c) *Completed test*

i) It may be applied to positive BGB tubes. Shake the tube, and streak with the help of a loop on the MacConkey agar plates as soon as possible in such a way so as to get discrete colonies. Incubate the plates at 37°C for 24 ± 2 hours.

ii) From each plate pick up typical or atypical colonies and inoculate lactose broth and nutrient agar slants. Incubate at 37°C for 24 to 48 hours.

iii) Nutrient agar slants can be used for gram—stain. If organisms are gram negative, non-spore forming bacilli and if gas is produced in laclose broth, the test is considered completed and the presence of coliform organisms is demonstrated.

iv) *Gram-stain technique* — Prepare a thin smear of the growth on the agar slant on a clean glass slide. Air dry, fix by passing the slide through a flame, and stain for 1 minute with ammonium oxalate—crystal violet solution. Wash the slide in water, immerse in Lugol's iodine solution for 1 minute. Wash the slide in water, blot dry; decolorize with ethyl alcohol for 30 seconds, using gentle agitation. Blot and cover with counter stain for 10 seconds with safranin, then wash, dry and examine under oil immersion.

Cells which decolorize and accept the safranin stain are pink in colour and defined as gram—negative in reaction. Cells which do not decolorize but retain the crystal violet stain, are deep blue in colour and are defined as gram-positive.

3.3.1.3 *Computing and recording of MPN* — The number of positive findings of coliform

group organisms (either presumptive, confirmed, or completed) resulting from the multiple portion decimal dilution planting should be computed as combination of the positives and recorded in terms of the Most Probable Number. The Most Probable Number for a variety of planting series and results is given in Appendix B.

3.3.2 *Membrane Filter (MF) Technique*

3.3.2.1 *Outline of the method* — The membrane filter technique in water analysis is becoming more and more popular due to its advantages over the multi tube dilution technique. Results are obtained within 24 or 48 hours as compared to 48 to 96 hours by multi tube dilution technique. Much larger volume and hence more representative sample can be tested. Results are obtained with much greater precision and require less laboratory space, equipment is not bulky and involves less labour. The limitations of this technique are few. Samples with high turbidity and less indicator bacterial count will be difficult to examine. Samples having high number of non-indicator organisms will give less count.

3.3.2.2 *Description of MF assembly*

a) There are many varieties of MF assemblies. The common one is millipore standard hydrosol filter holder. Most components are made of stainless steel. These are locking ring, fine mesh stainless steel screen for supporting the filter membrane and the funnel assembly.

b) Only those filter membranes may be used which have been found, through complete laboratory tests certified by manufacturer, to provide full bacterial retention, stability in use, freedom from chemicals inimical to the growth and development of bacteria, and satisfactory speed of filteration. They should preferably be gridmarked in such a way that bacterial growth is neither inhibited nor stimulated along the grid lines. The membrane filters of 47 mm in diameter and 0·45 micron pore size is used.

c) Absorbent pads for nutrients should consist of discs or filter paper or other materials known to be of high quality and free of sulphites or other substances that could inhibit bacterial growth. These should be approximately 45 mm in diameter and of thickness sufficient to absorb 1·8 to 2·2 ml of nutrient.

d) Sterilization of filter assembly, filters and absorbent pads is carried out at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0 5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. After sterilization, inmediately release steam in the autoclave by opening the outlet.

3.3.2.3 *Selection of sample size* — The size of the sample is governed by the expected bacterial density. An ideal quantity should result in the growth of 20 colonies and not more than 200 colonies of all types. Always, filter sample in duplicate. If water is heavily contaminated use less quantity of water. When less than 20 ml is to be filtered, dilute the portion to a minimum of 30 ml before filtration.

3.3.2.4 *Filtration of sample* — Using sterile forceps, place a sterile filter over the porous plate or stainless steel mesh of the apparatus, grid side up. Place the funnel unit carefully over the receptacle and lock it in place. Then pass the sample through the filter under vacuum. Rinse the filter by filtration, two to three times with 20 to 30 ml of sterile buffer water. Unlock the assembly, remove the filter by sterile forceps and place it on the sterile pad or agar with a rolling motion to avoid the entrapment of air.

3.3.2.5 *Medium* — Medium used for enumerating coliforms by membrane filter technique is known as M. Endo broth. The composition of the medium is given below:

Tryptone or polypeptone	10·0 g
Thiopeptone or thiotone	5·0 g
Casitone or Trypticase	5·0 g
Yeast extract	1·5 g
Lactose	12·5 g
Sodium chloride	5·0 g
Dipotassium hydrogen phosphate	4·375 g
Potassium dihydrogen phosphate	1·375 g
Sodium lauryl sulphate	0·05 g
Sodium desoxycholate	0·1 g
Sodium sulphite	2·1 g
Basic fuchsin	1·05 g

Dissolve the above ingredients in 1 000 ml of distilled water containing 20 ml of ethyl alcohol (95 percent). Heat the medium to boiling point. Do not heat for a long time or do not submit to steam under pressure. The final *p*H should be between 7.1 to 7.3.

3.3.2.6 *Procedure* — Saturate the pad in a small petridish with M. Endo broth and place the filter on it. Remove excessive medium by tilting. Invert the plates and incubate at 37°C for 24 hours under humid chamber. All colonies which produce a dark red colony with a metallic shine within 24 hour incubation are considered members of coliform group and

are counted. The count is made by a low power stereo microscope.

$$\text{Coliform density (coliforms/100 ml)} = \frac{\text{Coliform colonies counted} \times 100}{\text{Volume in ml of the sample filtered}}$$

3.3.3 *Test for Faecal Coliforms*

3.3.3.1 *General* — This procedure is used to differentiate coliforms of faecal origin from those of non-faecal origin. Faecal coliforms are those coliforms which can ferment lactose at 44·5°C within 24 ± 2 hours with the production of gas. Use brilliant green bile lactose broth medium for this test.

3.3.3.2 Subculture all presumptive positive tubes of the coliform test, at the end of 24 and 48 hours into BGB medium (*see* **3.3.1.1 c**) and incubate at 44·5°C for 24 hours in a water-bath. Gas formation within 24 hours is considered a positive reaction for faecal coliforms.

3.3.4 *Test for E. Coli*

3.3.4.1 E. Coli is one of the members of faecal coliforms which ferments lactose with the production of gas at 44·5°C within 24 hours, as well as produce indole from tryptophone at 44·5°C within 24 hours. Subculture from all the positive tubes of BGB broth at 44·5°C (faecal coliforms) into tubes of peptone water. Incubate at 44·5°C for 24 ± 2 hours. At the end of the incubation period, test for indole production by adding a few drops of Kovac's reagent. Positive test will give pink colour while negative test will give yellow colour.

3.3.5 *Delayed Incubation Method (Total coliforms)*

3.3.5.1 *General* — The delayed incubation MF method is useful in survey, monitoring or emergency situations when the single step coliform test cannot be performed at the sampling site or when time and temperature limits for sample storage cannot be met. The advantages are: a) The method permits confirmation and biochemical identification of organisms as necessary; and b) The method eliminates field processing and equipment needs.

The coliform bacteria can be kept for up to 72 hours with little effect on final counts. However, it is desirable that the holding period should be kept to the minimum. The applicability of the delayed incubation procedure for specific water source should be determined by comparative test procedures with conventional methods. The delayed incubation procedure is not a substitute for the immediate incubation test and should be used only when other alternatives are not applicable.

3.3.5.2 *Principle* — A specific volume of water sample is filtered through membrane filter. The filter retaining the micro-organisms is placed on an absorbent pad saturated with M-Endo preservative medium or M-Coliform Holding Broth in a tight-lidded petridish and transported from field site to the laboratory. The holding medium maintains the viability of the coliform organisms and generally does not permit visible growth during transport. In the laboratory the filter is transferred to M-Endo growth medium and incubated at 35°C for 18-24 h. Sheen colonies are counted as total coliforms/100 ml.

3.3.5.3 *Media*

a) M-Endo holding medium is prepared by adding 3·2 ml of solution benzoate solution (12 percent) per 100 ml of M-Coliform broth, prepared as given below. 4 ml of the cycloheximide solution may be added if required.

i) *M-Coliform broth*

Tryptone or polypeptone	10·0 g
Casitone or Trypticase	5·0 g
Lactose	12·5 g
Dipotassium hydrogen phosphate	4·375 g
Sodium lauryl sulphate	0·075 g
Basic fuchsin	1·05 g
Thiopeptone or thiotone	10·0 g
Yeast extract	1·5 g
Sodium chloride	5·0 g
Potassium dihydrogen phosphate	1·375 g
Sodium desoxycholate	0·10 g

Add 48 g of this medium to 1 litre of laboratory pure water containing 20 ml of 95 percent ethanol. Dentured alcohol should not be used. Heat in boiling water bath for solution. Store prepared medium in the dark at 4°C. Discard the unused medium after 96 hours.

ii) *Sodium banzoate solution* — Dissolve 12 g of sodium banzoate in about 85 ml of laboratory pure water, then bring to 100 ml final volume. Sterilize by autoclaving or filteration. Discard the solution after 6 months.

iii) *Cycloheximide solution (optional)* — Prepare an aqueous solution containing 1·25 grams of cycloheximine/100 ml laboratory pure water. Store solution in referigerator and discard after 6 months.

NOTE — Cycloheximide is used for samples that have shown problems of overgrowth with fungi. It is a powerful skin irritant and should be handled with care.

b) M-Coliform holding medium (LES holding medium) may be used as an alternative holding medium. To prepare the medium, add the following reagents in 1 litre of laboratory pure water and mix (do not heat) to dissolve:

Composition:

Tryptone or Trypticase Peptone	3·0 g
Dipotassium hydrogen phosphate	3·0 g
Sulphanilamide	1·0 g
Cycloheximide	0·5 g
M-Endo Broth MF	3·0 g
Sodium benzoate	1·0 g
Paraminobenzoic acid	1·2 g

3.3.5.4 *Procedure* — Saturate the sterile absorbent pads with about 2·0 ml of M-Endo Holding Medium or LES Holding Medium, prepared as outlined above. Pour off excess broth. Using a sterile forceps, place a membrane filter on the filter base, grid side up. Attach the funnel to the base of the filter unit; the membrane filter is now held between the funnel and base. Shake the sample vigorously about 25 times and measure into the funnel with the vacuum off. If the sample is less than 10 ml, add 10 ml of sterile dilution water to the membrane filter before adding the sample. The sample volume should be such as to produce counts of 20-80 coliform colonies. Filter the sample through the membrane and rinse the sides of the funnel walls at least twice with 20-30 ml sterile dilution water. Turn off the vacuum and remove the funnel from the base of the filter unit. With flame sterilized forceps remove the filter from the filterbase and place grid side up on an absorbent pad saturated with M-Endo Holding Medium of LES Holding Medium, using a rolling action at one edge. Exercise care to avoid trapping air bubbles under the membrane. Place top on petri dish and proceed with filtration of next volume. Clearly mark the lid of each petri dish, indicating location, time of collection, time of incubation, sample number and sample volume. Use a waterproof felt tip marker or grease pencil.

Inspect each membrane in the petri dish for uniform contact with the saturated pad. If air bubbles are present under the filter (indicated by bulges) remove filter with sterile forceps and roll onto the absorbent pad again. Seal the petri dish by firmly pressing down the top. Place the culture dish in shipping container and send it to the examining laboratory. At the examining laboratory remove the membrane from the holding medium. Place it in another dish containing M-Endo Broth or agar medium and complete testing as already described.

3.3.6 *Delayed Incubation Test for Faecal Coliforms*

3.3.6.1 The delayed incubation procedure for faecal coliforms is same as that for total coliforms, as given in **3.3.5** except that M-VFC holding medium is used. The composition of the medium is given below:

Casitone, Vitamin Free	0·2 g
Sodium benzoate	4·0 g
Sulphanilamide	0·5 g

Final *p*H 6·7 ± 0·2

Preparation — Add 4·7 g of medium (as given above) per litre of laboratory pure water containing 10 ml of 95 percent ethanol. Denatured alcohol should not be used. Heat slightly to dissolve the ingredients, then sterilize by membrane filtration (0·22 μm) Store prepared medium at 4°C. Discard after 1 month.

3.4 Test for Faecal Streptococci — The test terms faecal streptococci and enterococci have been used somewhat synonymously by many in recent years. The faecal streptococci group are indicators of faecal pollution of water because the general habitat of these organisms is the intestine of man and animals. They are gram positive cocci and ferment glucose with the production of acid only and are capable of growing in the presence of 40 percent bile and at 45°C. On the basis of newer concepts of speciation of faecal streptococci, it is suggested that the terms faecal streptococci and Lancefields group D streptococcus be considered synonymous. The standard test for the estimation of number of the faecal streptococci may be carried out either by the multiple tube dilution technique or by the membrane filter technique.

3.4.1 *Multiple Tube Dilution Technique* — Multiple tube dilution technique employs Presumptive test procedures and confirmed test procedures.

3.4.1.1 *Media*

a) *Dilution water — see* **3.2.3.2.**

b) *Azide dextrose broth (ADB)* — This is a presumptive test medium used for enumerating faecal streptococci in the water samples. Dissolves the following ingredients and adjust the *p*H to 7.3:

Tryptone or Polypeptone	15 g
Beef extract	4·5 g
Glucose	7·5 g
Sodium chloride	7·5 g
Sodium azide	0·2 g
Distilled water	1 000 ml

Dispense 6 to 7 ml of medium into 150 × 15 mm test tubes and plug with non-absorbent cotton. Sterilize in the autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. This a single strength medium and used for 1 ml aliquotes and decimal dilution when 10 ml sample or more has to be inoculated use double strength medium. This is prepared by using double the quantities given above in 1 000 ml of water. 10 ml of this double strength medium is put into each 150 × 18 mm test tube.

c) *Ethyl violet azide broth (EVA)* — Confirmatory medium used for enumerating faecal streptococci is as follows:

Tryptone or biosate	20 g
Glucose	5 g
Sodium chloride	5 g
Di-potassium hydrogen phosphate	2·7 g
Potassium di-hydrogen phosphate	2·7 g
Sodium azide	0·4 g
Distilled water	1 000 ml

Dissolve all the ingredients and adjust the *p*H to 7.1. Add 1 ml of 0·083 percent alcoholic solution of ethyl violet. Dispense 10 ml medium into 150 × 18 mm test tubes and plug with non-absorbent cotton. Sterilize in the autoclave at 1·02 ± 0 03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes.

3.4.1.2 *Procedure* — Shake the water sample thoroughly before making dilution or before inoculation.

a) *Presumptive test* — Inoculate a series of tubes of azide dextrose broth with appropriate graduated quantities of the water to be tested (follow the same procedure as given for coliforms). Incubate inoculated tubes at 37°C. Examine each tube at the end of 24 hours for the presence of turbidity. If no definite turbidity is present reinoculate and read at the end of 48 hours.

b) *Confirmed test* — All azide dextrose broth tubes showing turbidity after 24 or 48 hours incubation must be subjected to the confirmed test. Transfer three loopfuls of growth from each azide dexlrose broth to ethyl violet azide broth tubes. Incubate the inoculated tubes for 48 hours at 37°C. The presence of streptococci is indicated by the formation of a purple button at the bottom of the tube, or occassionally by a dense turbidity. Find out the MP value from Appendix B. Record the result as numbers per 100 ml of the sample.

3.4.2 *Membrane Filter Technique*

3.4.2.1 For outline of the method, description of the MF assembly, selection of the sample size and filtration of the sample, *see* **3.3.2**.

3.4.2.2 *Medium* — Medium used for enumeration of faecal streptococci by membrane filter technique is known as *M* enterococcus agar and is given below:

Tryptone	20 g
Yeast extract	5 g
Glucose	2 g
Di-potassium hydrogen phosphate	4 g
Sodium azide	0·4 g
Distilled water	1 000 ml.

Dissolve all the ingredients and adjust the *p*H to 7.2. Add 1 percent agar and heat sufficiently to dissolve agar. After slight cooling, add 1 ml of 1 percent sterile solution of 2, 3, 5 triphenyl tetrazolium chloride per 100 ml of the medium. Pour the plates (15 mm × 60 mm) by adding 10 ml of the agar. Allow to solidify and use fresh plates.

3.4.2.3 *Procedure* — Instead of pad, use a solid agar medium. Pour approximately 10 ml of *M* enterococcus agar into 60 mm petri dishes, Allow to harden and place the filter on it. Invert and incubate at 37°C for 48 hours under humid chamber. Count all red and pink colonies with the help of stereomicroscope. Express the count as number of faecal streptococci per 100 ml of water (*see* **3.3.2.6**).

NOTE — Since the requirement of medium for each sample is very little, dehydrated powder medium is recommended.

3.5 Test for Clostridium Welchii

3.5.1 *General* — Clostridium welchii are large rod-shaped, non-motile anaerobic bacteria which form spores which are relatively resistant to heat, drying and ordinary bacterial agents.

3.5.2 *Medium*

3.5.2.1 *Litmus milk medium* — Keep fresh raw milk of low bacterial content in the refrigerator for 18 ± 1 hours so that the cream may separate. Remove the cream and add 10 percent litmus solution to the milk to give a purplish blue colour. Distribute in tubes in 10 ml quantities. Add a mixture (melting point approximately 45°C) of equal parts of paraffin wax and petroleum jelly to form a layer about 2 to 5 mm thick of the surface of the medium. Steam for 30 minutes on 3 successive days. Test for sterility by incubation at 37·0° ± 0·5°C for 48 ± 3 hours.

3.5.3 *Procedure* — Inoculate varying quantities of the sample into bottles or tubes containing freshly boiled (in a water-bath) and rapidly cool litmus milk medium. Heat the tubes in a controlled water-bath at 80 ± 1°C for 15 minutes, remove from water bath, cool to approximately 37°C and place the tubes in an incubator at 37 ± 0·5°C for 5 days. Examine the tubes every day for signs of stormy fermentation which indicates a positive test. Further confirmation may be done by the spore staining technique.

3.5.4 *Confirmation by Spore Staining Technique*

3.5.4.1 *General* — Stormy fermentation is a presumptive indication for the presence of *clostridium welchii;* spore staining technique can be used for confirmation. The positive test eliminates doubt about non-spore forming rods and cocci and the position of the spore gives added information, for example, terminal, subterminal, central etc.

3.5.4.2 *Reagents*

a) *Ziehl Neelsen's Carbol Fuchsin (ZNCF)* — Its composition is given below:

Basic Fuchsin	5 g
Phenol	25 g
Ethanol (95 percent)	50 ml
Distilled water	500 ml

b) *Sulphuric acid* — 0·5 percent.

c) *Methylene blue* — 1 percent.

3.5.4.3 *Procedure* — Prepare a smear and fix it. Stain with ZNCF and heat the preparation until steam rises. Wash with water and treat with 0·5 percent sulphuric acid for 1 to 2 minutes. Wash with water and counter stain with 1 percent aqueous methylene blue for 3 minutes. Wash, dry, mount and observe under microscope. The cell will have bulging in the middle due to central spore. The spore stains are bright red and the protoplasm of bacilli blue.

3.6 Test for Iron Bacteria

3.6.1 *General* — Iron bacteria are considered to be capable of withdrawing iron present in their aqueous habitat and of depositing it in the form of hydrated ferric hydroxide on or in their mucilaginous secretion. Their presence may cause pitting and tuberculations in pipes and render the water unsuitable for domestic and industrial purposes. Bacteria of this type, to obtain energy, oxidize ferrous to ferric iron which is precipitated as ferric hydrate. Iron may be obtained from the pipe itself or from the water being carried. The amount of ferric hydrate deposited is very large in comparison with the enclosed cells. The main types are *Gallionella ferruginea, Leptothrix, Crenothrix Polyspora. Sphaerotilus natans and Thiobacillus ferrooxidants. Leptothrix* and *Crenothrix* are filamentous forms which deposit iron in their sheaths and the family *Gallionella* consists of stalked bacteria.

3.6.2 *Procedure* — It is generally sufficient to ascertain the presence or absence of these organisms in a water sample. For this purpose, the centrifuged deposit from the sample spread over a glass slide shall be examined under a microscope. The ferric deposits which may obscure the structure of the organisms may be removed by adding dilute hydrochloric acid to the smear on the slide and staining the organisms with Lugol's iodine solution (prepared by dissolving 1 g of iodine and 2 g of potassium iodide in 300 ml of water).

3.6.3 *Cultivation of Iron Bacteria* — When necessary the iron bacteria in the sample shall be cultivated and enriched by the method given in **3.6.3.1** and **3.6.3.2**.

3.6.3.1 *Gallionella*

a) *Medium* — Mix equal volumes of 10 percent solution of ferrous sulphate in water and 3 percent solution of agar at 45°C. Distribute in screw cap tubes and sterilize in the autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 20 minutes. Then prepare the agar slants. Dissolve 1·0 g of ammonium chloride, 0·5 g of dipotassium phosphate, 0·2 g of magnesium sulphate and 0·1 g of calcium chloride in 1 litre of water. Sterilize in the autoclave for 25 minutes at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately). Bubble carbondioxide through this medium for 10 to 15 seconds. Place in each agar tube a quantity of this liquid medium sufficient to cover the agar completely.

b) *Procedure* — Inoculate the tubes containing the medium with a drop of the suspension of the organisms in the centrifuged deposit of the sample and incubate at room temperature. Examine after 18 to 36 hours. White deposits on the sides of the culture tube indicate the presence of *Gallionella* colonies. Pick the deposit with a sterile needle, spread over a slide, and examine under the microscope as in **3.6.2**.

3.6.3.2 *Leptothrix*

a) *Medium* — Dissolve 1·0 g of ammonium sulphate, 0·5 g of magnesium sulphate, 0·1 g dibasic potassium phosphate, and 0·02 g of calcium nitrate in 1 litre of water. Place 100 ml quantities in conical flasks

and sterilize at 1·02 ± 0 03 kg/cm² gauge pressure (15 ± 0 5 psi gauge pressure, 120°C temperature approximately) in an autoclave for 20 minutes. Place in each flask 0 05 g of sterilized iron filings.

b) *Procedure* — Inoculate the medium prepared above with a suspension of the centrifuged deposit of the sample and incubate at room temperature. Examine after three days under the microscope as in **3.6.2**.

3.7 Test for Sulphate Reducing Bacteria

3.7 *General* — The sulphate reducing bacteria effect a direct reduction of sulphates. They are widely distributed in nature and are common in soils, canals and lake waters, sewage, marine sediment, etc. The water used for sealing petroleum or gas tanks generally harbour these bacteria. They may also be present in water cooling systems of industrial plants. These bacteria cause blackening of pulp in a paper mill, and corrode concrete sewage pipes and pipe surfaces. The most common of these organisms is *Desulphovibrio desulphuricans*. Some strains are mesophilic and grow best at 25 to 40°C while others are thermophilic and grow at 45 to 60°C. They are curved rods, and are strictly anaerobic.

3.7.2 *Apparatus*

3.7.2.1 *Culture bottle* — 60·0 ml glass stoppered bottle, sterilized in hot-air sterilizer.

3.7.3 *Medium* — Dissolve 10 g tryptone, 1 g sodium sulphite and 10 ml of 5 percent ferric citrate solution in 1 000 ml of distilled water. Sterile in an autoclave at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes.

3.7.4 Using multiple dilution techniquedistribute 10 ml of the medium in each test tube and sterilize at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 15 minutes. Add 1 ml of the sample or its dilution aseptically to each tube and cover the surface of the liquid by sterile liquid paraffin to a depth of 1 to 2 mm. Incubate the tube at 28 to 30°C for 2 to 3 days. The production of black colour in the medium will indicate the presence of sulphate reducing bacteria.

3.8 Test for Sulphur Bacteria

3.8.1 *General* — The organisms belonging to the group sulphur bacteria are autotrophic bacteria which oxidize elemental sulphur or reduce sulphur compounds, obtaining their carbon requirements from carbon dioxide. They are undesirable in water used in many ndustrial processes, and the acid produced during their metabolism may be destructive to concrete and other structures. *Thiobacillus thioparus* and *Thiobacillus thiooxidans* are the more common forms belonging to this group.

3.8.2 *Test for T. thioparus*

3.8.2.1 *Medium* — Dissolve 10·0 g of sodium thiosulphate pentahydrate, 2·0 g of dibasic potassium phosphate, 0·1 g of magnesium sulphate heptahydrate, 0·1 g of calcium chloride, 0·1 g of ammonium chloride, 0·02 g of ferric chloride hexahydrate and 0 02 g of manganese sulphate in 1 litre of water. Place 50 ml quantities in sterilized 250 ml conical flasks and sterilize by autoclaving at 1·02 ± 0·3 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately) for 10 minutes.

3.8.2.2 *Procedure* — Inoculate a shallow layer of the medium with a known volume of the sample (50 ml or 10 ml or less) and incubate at about 30°C for 2 to 3 days. In the presence of *T. thioparus* bacteria the surface of the inoculated medium becomes covered with sulphur from the autotrophic oxidation of the thiosulphate.

3.8.3 *Test for T. Thiooxidans*

3.8.3.1 *Medium* — Dissolve 0·2 g of ammonium sulphate, 0·5 g of magnesium sulphate heptahydrate, 3·0 g of monobasic potassium phosphate, 0·25 g of calcium chloride and 0 01 g of ferrous sulphate in 1 litre of water. Weigh 1·0 g of elemental sulphur in a 250-ml flask and add to it 100 ml of this solution.

NOTE — Ferrous sulphate solution should be sterilized by filtration through a millipore membrane filter of 0·45 µm and then mixed aseptically to the rest of the basal medium. Heating, steaming or autoclaving of ferrous sulphate solution may result in oxidation and hydrolysis of the salt. Solution of potassium phosphate should be sterilized separately and then added aseptically to the basal medium. Sulphur should be sterilized by steaming for 30 minutes on three successive days before adding to the medium Rest of the medium should be sterilized in a steam sterilizer for 30 minutes on 3 successive days.

3.8.3.2 *Procedure* — Inoculate flasks containing the medium with 10 ml or less of the sample, and incubate at 25 to 30°C for 4 to 5 days. The sulphur sinks to the bottom, the reaction of the medium decreases to *p*H 2·0 and in the presence of T. thiooxidans the medium will become turbid.

3.9 Test for Gelatin Liquefying Bacteria

3.9.1 *General* — Certain micro-organisms are capable of producing proteolytic ferments which digest and liquefy gelatin. The presence of these organisms in process water is of importance in industries such as the manufacture of photographic films, edible gelatin, glue and in food processing.

3.9.2 *Medium* — Dissolve 3 g of beef extract, 5 g of peptone and 120 g of gelatin in 1 litre of water over a water-bath. Cool to about 50°C and distribute into tubes in quantities of 10 to 15 ml. Sterilize in the autoclave for 20 minutes at 1·02 ± 0·03 kg/cm² gauge pressure (15 ± 0·5 psi gauge pressure, 120°C temperature approximately). The final reaction of the medium shall be *p*H **6.8**.

3.9.3 *Procedure*

3.9.3.1 Place 1 ml of the well shaken sample in a sterile petri dish. Add the molten medium at 30·0 ± 0·2°C and mix thoroughly by careful rotation and to and fro movement of the dish placed on a flat table. Not more than 20 minutes shall elapse between placing the sample in the petri dish and adding the medium. Incubate the dish at 20·0 ± 0·2°C and examine every day for seven days for evidence of liquefaction of the medium around the colonies of micro-organisms. If the atmospheric temperature is above 20°C the examination of the dish should be made as soon as it is taken from the incubator before the media starts thawing. The negative dishes shall be incubated further up to a period of at least 21 days. It is preferable to inoculate at least 5 dishes for each sample.

3.9.3.2 *Confirmation* — If further confirmation of the gelatin liquefying property of the organisms is desired, the following procedure shall be adopted:

a) *Preparation of nutrient gelatin medium* — Dissolve 3 g of beef extract, 5 g of sodium chloride and 10 g of peptone in 1 litre of water. To this solution, add 120 g of gelatin, dissolve in a water-bath, cool to about 50°C and adjust to *p*H **7.5**. Add 10 g of egg albumen, mix thoroughly, heat for half an hour and filter while hot. Distribute in 10 ml quantities in previously autoclaved tubes and sterilize by steaming for 20 minutes on 3 successive days.

b) Place the gelatin medium tubes at 20·0 ± 0·2°C for the medium to harden and take out only just before use. Pick out a suspected colony from the petri dish culture obtained in **3.9.3.1** using a straight needle and prepare a stab inoculation in the tube. Incubate the tubes at 20.0 ± 0·2°C and examine for evidence of liquefaction as in **3.9.3.1**.

3.9.3.3 If a 20°C incubator is not available, the test for presence of gelatin liquefying organisms may be carried out by inoculating 1 ml of the sample into a tube of nutrient gelatin medium and incubating at 37·0 ± 0·2°C. Examine every day for seven days for evidence of liquefaction by placing the tube in cooled water (below 20°C) and observing whether the medium hardens or not. An uninoculated tube of nutrient gelatin medium shall also be incubated and tested as a control.

3.10 Test for Slime Forming Bacteria

3.10.1 *General* — A large variety of non-pathogenic bacteria is carried by water which is of vital interest to bacteriologists and water engineers because these may produce slime which will adhere to structures and increase either by growth or by collecting and holding insoluble debris from water supply. The presence of these slime forming organisms is especially undesirable to condenser systems, paper mills, food processing plants, etc. These are mixtures of various types of bacteria. The time and labour involved in making bacterial isolations and counts on laboratory media from industrial slimes may not, however, be justified by the information gained. A clear picture of the slime may be obtained by direct microscopic examination of the material.

3.10.2 *Procedure*

3.10.2.1 Place on a clear glass slide a small amount of the sample and spread it evenly. Cover with a cover glass and examine under the low power of the microscope for large forms, such as algae and mould and record.

3.10.2.2 Prepare another mount as above and stain with Lugol's iodine solution (*see* **3.6.2**). Examine under the high power of the microscope for filamentous bacteria. Dry and fix the smear, stain by the Gram method and observe under the oil immersion. A variety of organisms may be observed of which one or two types may be prominent. Record the observations.

4. MICROSCOPIC EXAMINATION

4·0 Outline of the Method — The sample shall be examined microscopically as early as possible after collection. Qualitative assessment by identifying the organisms shall be made before preserving the sample. Quantitative assessment of the individual organisms may be made after preservation. In general indentification of the organisms up to their generic names is enough. Further identification to species level is only essential under special circumstances and can only be done by a specially trained personnel. Quantitative examination by direct counting can be done if organisms are sufficiently numerous. Otherwise the organisms should be concentrated in a small volume of the sample as prescribed in **4.1**.

4.1 Concentration of the Organisms

4.1.1 *The Sedgwick Rafter Method*

4.1.1.1 *Equipment*

a) *Filter funnel* — Cylindrical, with diameter 5 cm at the top, a straight side for about 20 cm and narrowing over a distance of about 5 cm to a bore of 1 cm diameter and terminating in a straight portion of 1 cm diameter about 6 cm in length. The capacity shall be about 500 ml. A single-holed rubber stopper shall be fitted tightly into the bottom. A small glass U-tube is inserted in the stopper with the outer end extending about 0·2 cm above the inner end of the stopper.

b) *Filtering sand* — Washed white sand, passing 250 micron IS Sieve and retained on 125-micron IS Sieve.

c) *Cloth discs* — About 1 cm in diameter. These shall preferably be cut from bolting silk cloth having 80 meshes/cm², alternately nylon or linen cloth may be used.

4.1.1.2 *Procedure* — Place a cloth disc after moistening it on the rubber stopper of the funnel and insert the stopper firmly in the lower end of the funnel. Introduce a small amount of filtering sand into the funnel to form a layer not less than 12 mm thick on the top of the rubber stopper. Place the funnel in an upright position in a suitable support, introduce a small quantity of water for settling the sand and allow it to drain through the sand. Mix the sample under test gently but thoroughly and add a measured portion (500 to 1 000 ml) to the funnel without disturbing the sand. This is done by commencing the addition of the sample before all the liquid has drained through. Continue filtration of the sample, returning the first 100 ml to the funnel, washing down the sides of the funnel with a stream of the filtrate from time to time for dislodging any organisms adhering to the sides, until the water level reaches that of the outer end of the U-tube. Then remove the U-tube and allow the remaining liquid to drain completely. Place a small beaker under the funnel and remove the stopper, catching the sand in the beaker. Flush the inside wall of the funnel with 5 to 10 ml of 3 to 5 percent formalin in water collecting it in the beaker. Shake the beaker gently for separating the organisms from the sand particles, allow time for the sand particles to settle, and decant the suspension of the organisms into a second beaker. Wash the sand with 5 ml of dilute formalin and decant into the second beaker as before. Measure the total volume of the concentrate and make up to a known volume, usually a multiple of five,

$$\text{Degree of concentration} = \frac{V_1}{V_2}$$

where

V_1 = volume in ml of sample filtered, and

V_2 = volume in ml of the concentrate.

4.1.2 *Centrifuge Method*

4.1.2.1 *Equipment*

a) *Centrifuge* — Electrically operated, holding 20 ml tubes and with a speed of 2 500 to 3 000 rev/min.

b) *Centrifuge tubes* — 10 ml capacity with the lower end tapered and graduated.

4.1.2.2 *Procedure* — Fill the centrifuge tubes to the mark with the well mixed sample and centrifuge at 2 500 to 3 000 rev/min for 10 minutes. All matter in suspension is driven to the bottom of the tubes. Carefully pour off the clear supernatant liquid up to a little above the 0·1 ml mark in the narrow portion of the tube, taking care not to disturb the sediment. Remove the liquid to exactly the level of the 0·1 ml mark with a pipette and after expelling the liquid, use the pipette for mixing the deposit in the tube thoroughly with the remaining liquid. Pool the concentrates thus obtained in the centrifuge tubes and make up to a known volume. Add a trace of formalin for preventing the movement of mobile organisms that may be present.

4.1.2.3 If the organisms in the original sample are few in number, it will be necessary to use a large volume of the sample for obtaining a suitable concentrate. For this purpose a Foerst type of continuous centrifuge having a speed of 20 000 rev/min may be used. In this apparatus the sample is fed continuously into a revolving bowl and the organisms are deposited in the angle formed by the junction of the side wall of the bowl with the bottom. After centrifugation, the compacted deposit is gently brushed loose from the bowl, mixed with the small amount of water in the bowl and transferred to a beaker and made up to a known volume.

4.1.3 *Millipore Filter*

4.1.3.1 Phytoplankton concentration may be made through filtering a known volume through a millipore filter assembly and the number of cells trapped on the filter paper may be counted by direct observation with a compound microscope.

4.1.4 In case of heavy water samples having high phytoplankton count initial concentration of the sample is not necessary. However, known volume may be strained through the plankton net into a collection vessel for zooplankton assessment.

4.2 Procedure for Enumeration

4.2.1 *Equipment*

4.2.1.1 *Compound microscope* — Provided with 7·5 × and 10 × oculars and 16 mm objective shall be used. A binocular microscope is preferable.

4.2.1.2 *Whipple ocular micrometer* — It is a circular glass disc which fits exactly into the interior of the ocular. It shall bear an accurately ruled square subdivided into 25 equal squares. One of the small squares in the centre will be further subdivided into 25 smaller squares.

4.2.1.3 *Stage micrometer* — It consists of a thin glass disc mounted permanently upon a glass slide. An accurate linear scale, usually 1 mm divided into hundredths, is etched on the underside of the disc.

4.2.1.4 *Counting cell* — It is a glass or brass rectangle, 50 × 20 × 1 mm, sealed to a glass microscope slide. It has a capacity of 1 ml and encloses an area of 1 000 mm². A rectangular cover glass large enough to cover the cell is required.

4.2.2 *Procedure*

4.2.2.1 *Standardization of the Whipple micrometer* — Place the micrometer ruling side downwards in the ocular of the microscope. Place the stage micrometer on the stage of the microscope and, using the 16 mm objective, focus to get a clear image of the rulings. Adjust the draw tube of the microscope so that one side of the ocular micrometer covers exactly 1 mm on the stage micrometer. Record the tube length of the microscope for future reference. In this position, the area enclosed by the large square of the ocular micrometer is 1 mm² on the stage, that enclosed by each of the 100 interior squares is 0·01 mm² and that enclosed by one of the smallest squares is 0·000 4 mm². One side of the smallest square is 0·02 mm or 20 μm in length.

4.2.2.2 Place the counting cell on a level surface. Shake the concentrated sample (*see* **4.1.1** or **4.1.2**) in the beaker gently but thoroughly and with a pipette, transfer the sample to the counting cell filling it completely. Place a clean cover glass over the cell by sliding it gently from one end taking care not to enclose any air bubble. Allow five minutes for the organisms to settle and examine the cell under the microscope equipped with the calibrated Whipple ocular micrometer. Select at random one area which is entirely within the cell and count all the organisms within the large square of the ocular micrometer. If an organisms is partly within and partly outside the counting square, estimate and record the fractional portion within the square. The microscope shall be focussed through the entire depth of the cell for detecting and counting all the organisms. Count also particles of debris and other matter and record them under that heading. Count the organisms in ten fields moving the cell appropriately. Record the number of organisms of each kind counted in a suitable form. No special form is prescribed but in general, the form used should provide space for recording information about the source of the sample, time and date of collection, date of the examination, laboratory serial number, and name of the examiner. It shall be ruled to facilitate entry of results when ten fields are counted.

4.2.3 *Calculation* — Calculate the number of organisms per millilitre of the original sample as follows:

$$\text{Number of organisms/ml of original sample} = \frac{1\,000\,V_1 \times N}{V \times n}$$

where

V_1 = volume in ml of concentrated sample taken for the test,

N = total number of organisms counted,

V = volume in ml of original sample taken for concentration and filtered, and

n = number of fields counted.

4.2.3.1 The value of the expression $\frac{1\,000\,V_1}{V}$ is commonly referred to as the Sedgwick Rafter factor.

4.2.3.2 *Reporting in cubic standard units* — For most purposes of biological examination reporting in terms of number of organisms of each kind in 1 ml of the sample will be sufficient. When the size of the organisms has also to be estimated, the system of reporting in cubic standard units shall be adopted, the cubic standard unit is 0·000 008 mm³ or 8 000 μm³, the length of one side of which is 0·02 mm. For obtaining the volume of the organisms, the microscope shall be equipped with a graduated micrometer head and vernier on the fine adjustment. Measure the length and breadth of each organisms at the time the count is made, with the aid of the ocular micrometer, noting the squares or partial squares which the organisms cross. One side of the square is 0·02 mm. Focus carefully the topmost and bottom most surface of the organisms and determine its thickness from the vertical distance through which the objective moved by reading on the graduated fine adjustment screw. Organisms which are uniform in size, such as diatoms, may be counted individually and then converted to volume by multiplying the total number counted by a constant factor expressing their size. Calculate the total volume of the organisms of kind per millilitre of the original sample and express the result as cubic standard unit per millilitre.

4.2.4 *Drop Method for Counting Phytoplankton*

4.2.4.1 Direct microscopic examination is necessary for qualitative enumeration and for further quantitative assessment of phytoplankton in terms of cell numbers. Since delicate cells such as small flagellates are almost unrecognisable in a fixed state a direct examination of the sample in fresh condition is ideal, if necessary after centrifugation.

4.2.4.2 *Procedure* — Take a drop, small enough to fit under a square cover glass of 22 mm size and put on a clean microscopic slide. Standardize the drop with a long drawn, preferably with a pasture pipette and always use a uniform sized drop. To minimize experimental error in drop selection count more number of drops for obtaining uniformity in cell count per drop. After placing the drop on a clean slide drop gently a 22 mm square cover glass. Take care that there are no air bubbles under the cover glass. Place the slide on the microscope stage and bring the middle of one edge of the cover glass into view with high power objective. Move the slide from one side to the other counting the number of cells in that transect. Carry out this operation quickly so as to avoid drying of the drop and consequent formation of air cavities under the cover glass. Repeat the operation, counting another transect well separated from the first. Count another drop in the same way. If the results are very different from the first, count a third drop. Repeat this till uniform count is achieved. Calculate the number of cells per drop using the following formula:

$$\text{Total number cells/drop} = \frac{\text{Area of the cover glass} \times \text{individual counts recorded per transect}}{\text{Area of one transect}}$$

Count per ml can be calculated by multiplying the count/drop with number of such drops that make one millilitre.

APPENDIX A

(*Clause* 2.1.5)

PARTICULARS TO BE SUPPLIED ALONG WITH SAMPLES

A-1. While submitting samples, the following particulars shall be supplied along with sample:

a) Name and address of person requesting the examination;

b) Name, designation or other identification particulars of the person drawing samples;

c) Date and time of collection and despatch;

d) Reasons for examination and whether it is a routine sample or otherwise;

e) Source of water (well, spring, stream, public supply, etc);

f) Exact place from which sample was taken. If from a tap whether the sample was drawn through a cistern, or directly from the mains;

g) The method of purification and sterilization used, if any; details of dose of chemicals, point of application, quantity treated, etc;

h) Temperature of the sample;

j) Weather at the time of collection and particulars of recent rainfall;

k) Whether the water becomes affected in appearance, odour or taste after heavy rains;

m) If the sample has been taken from a well, then:

1) Depth of well, and of water surface from ground level;

2) Whether covered or uncovered, and nature, material, and construction of the cover;

3) Whether newly constructed or with any recent alterations which might affect the condition of the water;

4) Type of construction — i) bricks set dry or in cement; ii) cement or cylinder lined, and whether puddled outside the lining; iii) depth of lining; iv) whether bricked above ground surface, if so, height of coping; v) presence and extent of apron; and vi) method of pumping or other means of raising water;

5) Proximity of drains, cesspools or other possible sources of pollution, and distance from source;

6) Any discoloration of the sides of the well or other visible indication of pollution;

7) Nature of subsoil and water-bearing stratum.

NOTE — When available, a section or drawing of the well and general surroundings should be furnished.

n) If the sample has been taken from a spring, then:
   1) Stratum from which it issues;
   2) Whether the sample has been taken direct from the spring or from a collecting chamber. If from the latter, the mode of construction of chamber;

p) If the sample has been taken from a river or stream, then:
   1) Depth below surface at which the sample was taken;
   2) Whether the sample was taken from the middle or side;
   3) Whether the level of water is above or below the average;
   4) Conditions of weather at the time of sampling and particulars of any recent rainfall or flood conditions;
   5) Observations with reference to any possible sources of pollution in the vicinity and approximate distance from sampling point; and

q) Results of field tests made on the sample.

APPENDIX B

(*Clause* 3.3.1.3)

TABLES OF MOST PROBABLE NUMBERS

TABLE 1 MOST PROBABLE NUMBER (MPN) OF ORGANISMS PER 100 ml OF SAMPLE AND CONFIDENCE LIMITS USING 1 TUBE OF 50 ml AND 5 TUBES OF 10 ml

Number of Positive Tubes		Most Probable Number (MPN) per 100 ml	Limits Within Which MPN Per 100 ml Can Lie	
50-ml Tubes	10-ml Tubes		Lower Limit	Upper Limit
(1)	(2)	(3)	(4)	(5)
0	1	1	<0·5	4
0	2	2	<0·5	6
0	3	4	<0·5	11
0	4	5	1	13
1	0	2	<0·5	6
1	1	3	<0·5	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40

TABLE 2 MOST PROBABLE NUMBER (MPN) OF ORGANISMS PER 100 ml OF SAMPLE AND CONFIDENCE LIMITS USING 1 TUBE OF 50 ml, 5 TUBES OF 10 ml AND 5 TUBES OF 1 ml

Number of Positive Tubes			Most Probable Number (MPN) Per 100 ml	Limits Within Which MPN Per 100 ml Can Lie	
50-ml Tubes	10-ml Tubes	1-ml Tubes		Lower Limit	Upper Limit
(1)	(2)	(3)	(4)	(5)	(6)
0	0	1	1	<0·5	4
0	0	2	2	<0·5	6
0	1	0	1	<0 5	4
0	1	1	2	<0 5	6
0	1	2	3	<0·5	8
0	2	0	2	<0·5	6
0	2	1	3	<0·5	8
0	2	2	4	<0·5	11
0	3	0	3	<0·5	8
0	3	1	5	<0·5	13
0	4	0	5	<0·5	13
1	0	0	1	<0·5	4
1	0	1	3	<0·5	8
1	0	2	4	<0·5	11
1	0	3	6	<0·5	15
1	1	0	3	<0·5	8
1	1	1	5	<0·5	13
1	1	2	7	1	17
1	1	3	9	2	21
1	2	0	5	<0·5	13
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	18	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	69
1	4	3	28	8	85
1	4	4	35	12	101
1	4	5	43	15	117
1	5	0	24	8	75
1	5	1	35	12	101
1	5	2	54	18	138
1	5	3	92	27	217
1	5	4	161	39	450

TABLE 3 MOST PROBABLE NUMBER (MPN) OF ORGANISMS PRESENT PER 100 ml OF SAMPLE AND CONFIDENCE LIMITS USING 5 TUBES OF 10 ml, 5 TUBES OF 1 ml AND 5 TUBES OF 0·1 ml

Number of Positive Tubes			Most Probable Number (MPN) Per 100 ml	Limits Within Which MPN Per 100 ml Can Lie	
10-ml Tubes	1-ml Tubes	0·1-ml Tubes		Lower Limit	Upper Limit
(1)	(2)	(3)	(4)	(5)	(6)
0	0	1	2	<0·5	7
0	0	2	4	<0·5	11
0	1	0	2	<0·5	7
0	1	1	4	<0·5	11
0	1	2	6	<0·5	15
0	2	0	4	<0·5	11
0	2	1	6	<0·5	15
0	3	0	6	<0·5	15
1	0	0	2	<0·5	7
1	0	1	4	<0·5	11
1	0	2	6	<0·5	15
1	0	3	8	1	19
1	1	0	4	<0·5	11
1	1	1	6	<0·5	15
1	1	2	8	1	19
1	2	0	6	<0·5	15
1	2	1	8	1	19
1	2	2	10	2	23
1	3	0	8	1	19
1	3	1	10	2	23
1	4	0	11	2	25
2	0	0	5	<0·5	13
2	0	1	7	1	17
2	0	2	9	2	21
2	0	3	12	3	28
2	1	0	7	1	17
2	1	1	9	2	21
2	1	2	12	3	28
2	2	0	9	2	21
2	2	1	12	3	28
2	2	2	14	4	34
2	3	0	12	3	28
2	3	1	14	4	34
2	4	0	15	4	37
3	0	0	8	1	19
3	0	1	11	2	25
3	0	2	13	3	31
3	1	0	11	2	25
3	1	1	14	4	34
3	1	2	17	5	46
3	1	3	20	6	60
3	2	0	14	4	34
3	2	1	17	5	46
3	2	2	20	6	60
3	3	0	17	5	46
3	3	1	21	7	63
3	4	0	21	7	63
3	4	1	24	8	72
3	5	0	25	8	75
4	0	0	13	3	31
4	0	1	17	5	46
4	0	2	21	7	63
4	0	3	25	8	75

(*Continued*)

TABLE 3 MOST PROBABLE NUMBER (MPN) OF ORGANISMS PRESENT PER 100 ml OF SAMPLE AND CONFIDENCE LIMITS USING 5 TUBES OF 10 ml, 5 TUBES OF 1 ml AND 5 TUBES OF 0·1 ml — *Contd*

Number of Positive Tubes			Most Probable Number (MPN) Per 100 ml	Limits Within Which MPN Per 100 ml Can Lie	
10-ml Tubes	1-ml Tubes	0·1-ml Tubes		Lower Limit	Upper Limit
(1)	(2)	(3)	(4)	(5)	(6)
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	2	2	32	11	91
4	3	0	27	9	80
4	3	1	33	11	93
4	3	2	39	13	106
4	4	0	34	12	96
4	4	1	40	14	108
4	5	0	41	14	110
4	5	1	48	16	124
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	114
5	0	3	58	19	144
5	0	4	76	24	180
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	154
5	1	3	84	26	197
5	2	0	49	17	126
5	2	1	70	23	168
5	2	2	94	28	219
5	2	3	120	33	281
5	2	4	148	38	366
5	2	5	177	44	515
5	3	0	79	25	187
5	3	1	109	31	253
5	3	2	141	37	343
5	3	3	175	44	503
5	3	4	212	53	669
5	3	5	253	77	788
5	4	0	130	35	302
5	4	1	172	43	486
5	4	2	221	57	698
5	4	3	278	90	849
5	4	4	345	117	999
5	4	5	426	145	1 161
5	5	0	240	68	754
5	5	1	348	118	1 005
5	5	2	542	180	1 405

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'.

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 110002
Telephones: 323 01 31, 323 3375, 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 700054	337 84 99, 337 85 61 337 86 26, 337 91 20
Northern	: SCO 335-336, Sector 34-A, CHANDIGARH 160022	60 38 43 60 20 25
Southern	: C.I.T. Campus, IV Cross Road, CHENNAI 600113	254 12 16, 254 14 42 254 25 19, 254 13 15
Western	: Manakalaya, E9 MIDC, Marol, Andheri (East) MUMBAI 400093	832 92 95, 832 78 58 832 78 91, 832 78 92

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

Printed at Simco Printing Press, Delhi