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पीने का पानी — विशिष्टि
(दूसरा पुनरीक्षण)

Indian Standard
DRINKING WATER — SPECIFICATION
(*Second Revision*)

ICS 13.060.20

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BUREAU OF INDIAN STANDARDS
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**AMENDMENT NO. 1 JUNE 2015
TO
IS 10500 : 2012 DRINKING WATER — SPECIFICATION**

(Second Revision)

[Page 2, Table 2, Sl No. xii), col 3] — Substitute '1.0' for '0.3'.

[Page 3, Table 3, Sl No. x), col 4] — Substitute 'No relaxation' for '0.05'.

(FAD 14)

Publication Unit, BIS, New Delhi, India

AMENDMENT NO. 2 SEPTEMBER 2018
TO
IS 10500 : 2012 DRINKING WATER — SPECIFICATION
(Second Revision)

(Page 1, clause 4.1.1, line 6) — Substitute ‘IS 15185’ for ‘IS 1622’.

[Page 2, Table 2, Sl No. (v), col 4] — Substitute ‘2.4’ for ‘1.0’.

(FAD 14)

Publication Unit, BIS, New Delhi, India

FOREWORD

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

This standard was originally published in 1983. A report prepared by the World Health Organization in cooperation with the World Bank showed that in 1975, some 1 230 million people were without safe water supplies. These appalling facts were central to the United Nations decision to declare an International Drinking Water Supply and Sanitation decade, beginning in 1981. Further, the VI Five-Year Plan of India had made a special provision for availability of safe drinking water for the masses. Therefore, the standard was formulated with the objective of assessing the quality of water resources, and to check the effectiveness of water treatment and supply by the concerned authorities.

The first revision was undertaken to take into account the up-to-date information available about the nature and effect of various contaminants as also the new techniques for identifying and determining their concentration. Based on experience gained additional requirements for alkalinity; aluminium and boron were incorporated and the permissible limits for dissolved solids, nitrate and pesticides residues modified.

As per the eleventh five year plan document of India (2007-12), there are about 2.17 lakh quality affected habitations in the country with more than half affected with excess iron, followed by fluoride, salinity, nitrate and arsenic in that order. Further, approximately, 10 million cases of diarrhoea, more than 7.2 lakh typhoid cases and 1.5 lakh viral hepatitis cases occur every year a majority of which are contributed by unclean water supply and poor sanitation. The eleventh five year plan document of India (2007-2012) recognizes dealing with the issue of water quality as a major challenge and aims at addressing water quality problems in all quality affected habitations with emphasis on community participation and awareness campaigns as well as on top most priority to water quality surveillance and monitoring by setting up of water quality testing laboratories strengthened with qualified manpower, equipments and chemicals.

The second revision was undertaken to upgrade the requirements of the standard and align with the internationally available specifications on drinking water. In this revision assistance has been derived from the following:

- a) EU Directives relating to the quality of water intended for human consumption (80/778/EEC) and Council Directive 98/83/EC.
- b) USEPA standard — National Primary Drinking Water Standard. EPA 816-F-02-013 dated July, 2002.
- c) WHO Guidelines for Drinking Water Quality. 3rd Edition Vol. 1 Recommendations, 2008.
- d) Manual on Water Supply and Treatment, third edition — revised and updated May 1999, Ministry of Urban Development, New Delhi.

This standard specifies the acceptable limits and the permissible limits in the absence of alternate source. It is recommended that the acceptable limit is to be implemented as values in excess of those mentioned under 'Acceptable' render the water not suitable. Such a value may, however, be tolerated in the absence of an alternative source. However, if the value exceeds the limits indicated under 'permissible limit in the absence of alternate source' in col 4 of Tables 1 to 4, the sources will have to be rejected.

Pesticide residues limits and test methods given in Table 5 are based on consumption pattern, persistence and available manufacturing data. The limits have been specified based on WHO guidelines, wherever available. In cases where WHO guidelines are not available, the standards available from other countries have been examined and incorporated, taking in view the Indian conditions.

In this revision, additional requirements for ammonia, chloramines, barium, molybdenum, silver, sulphide, nickel, polychlorinated biphenyls and trihalomethanes have been incorporated while the requirements for colour, turbidity, total hardness, free residual chlorine, iron, magnesium, mineral oil, boron, cadmium, total arsenic, lead, polynuclear aromatic hydrocarbons, pesticides and bacteriological requirements have been modified.

In this revision, requirement and test method for virological examination have been included. Further, requirements and test methods for cryptosporidium and giardia have also been specified.

Routine surveillance of drinking water supplies should be carried out by the relevant authorities to understand the risk of specific pathogens and to define proper control procedures. The WHO Guidelines for Drinking Water Quality, 3rd Edition, Vol. 1 may be referred for specific recommendations on using a water safety approach incorporating risk identification. Precautions/Care should be taken to prevent contamination of drinking water from chlorine resistant parasites such as cryptosporidium species and giardia.

Indian Standard

DRINKING WATER — SPECIFICATION

*(Second Revision)***1 SCOPE**

This standard prescribes the requirements and the methods of sampling and test for drinking water.

2 REFERENCES

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

3 TERMINOLOGY

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

3.1 Drinking Water — Drinking water is water intended for human consumption for drinking and cooking purposes from any source. It includes water (treated or untreated) supplied by any means for human consumption.

4 REQUIREMENTS

Drinking water shall comply with the requirements given in Tables 1 to 4. The analysis of pesticide residues given in Table 3 shall be conducted by a recognized laboratory using internationally established test method meeting the residue limits as given in Table 5.

Drinking water shall also comply with bacteriological requirements (*see 4.1*), virological requirements (*see 4.2*) and biological requirements (*see 4.3*).

4.1 Bacteriological Requirements**4.1.1 Water in Distribution System**

Ideally, all samples taken from the distribution system including consumers' premises, should be free from coliform organisms and the following bacteriological quality of drinking water collected in the distribution system, as given in Table 6 is, therefore specified when tested in accordance with IS 1622.

4.2 Virological Requirements

4.2.1 Ideally, all samples taken from the distribution

Table 1 Organoleptic and Physical Parameters*(Foreword and Clause 4)*

| SI No. | Characteristic | Requirement (Acceptable Limit) | Permissible Limit in the Absence of Alternate Source | Method of Test, Ref to Part of IS 3025 | Remarks |
|--------|--|--------------------------------|--|--|--|
| (1) | (2) | (3) | (4) | (5) | (6) |
| i) | Colour, Hazen units, <i>Max</i> | 5 | 15 | Part 4 | Extended to 15 only, if toxic substances are not suspected in absence of alternate sources |
| ii) | Odour | Agreeable | Agreeable | Part 5 | a) Test cold and when heated b) Test at several dilutions |
| iii) | pH value | 6.5-8.5 | No relaxation | Part 11 | — |
| iv) | Taste | Agreeable | Agreeable | Parts 7 and 8 | Test to be conducted only after safety has been established |
| v) | Turbidity, NTU, <i>Max</i> | 1 | 5 | Part 10 | — |
| vi) | Total dissolved solids, mg/l, <i>Max</i> | 500 | 2 000 | Part 16 | — |

NOTE — It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 2 General Parameters Concerning Substances Undesirable in Excessive Amounts
(Foreword and Clause 4)

| Sl No. | Characteristic | Requirement (Acceptable Limit) | Permissible Limit in the Absence of Alternate Source | Method of Test, Ref to | Remarks |
|--------|--|--------------------------------|--|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) |
| i) | Aluminium (as Al), mg/l, <i>Max</i> | 0.03 | 0.2 | IS 3025 (Part 55) | — |
| ii) | Ammonia (as total ammonia-N), mg/l, <i>Max</i> | 0.5 | No relaxation | IS 3025 (Part 34) | — |
| iii) | Anionic detergents (as MBAS) mg/l, <i>Max</i> | 0.2 | 1.0 | Annex K of IS 13428 | — |
| iv) | Barium (as Ba), mg/l, <i>Max</i> | 0.7 | No relaxation | Annex F of IS 13428* or IS 15302 | — |
| v) | Boron (as B), mg/l, <i>Max</i> | 0.5 | 1.0 | IS 3025 (Part 57) | — |
| vi) | Calcium (as Ca), mg/l, <i>Max</i> | 75 | 200 | IS 3025 (Part 40) | — |
| vii) | Chloramines (as Cl ₂), mg/l, <i>Max</i> | 4.0 | No relaxation | IS 3025 (Part 26)* or APHA 4500-Cl G | — |
| viii) | Chloride (as Cl), mg/l, <i>Max</i> | 250 | 1 000 | IS 3025 (Part 32) | — |
| ix) | Copper (as Cu), mg/l, <i>Max</i> | 0.05 | 1.5 | IS 3025 (Part 42) | — |
| x) | Fluoride (as F) mg/l, <i>Max</i> | 1.0 | 1.5 | IS 3025 (Part 60) | — |
| xi) | Free residual chlorine, mg/l, <i>Min</i> | 0.2 | 1 | IS 3025 (Part 26) | To be applicable only when water is chlorinated. Tested at consumer end. When protection against viral infection is required, it should be minimum 0.5 mg/l |
| xii) | Iron (as Fe), mg/l, <i>Max</i> | 0.3 | No relaxation | IS 3025 (Part 53) | Total concentration of manganese (as Mn) and iron (as Fe) shall not exceed 0.3 mg/l |
| xiii) | Magnesium (as Mg), mg/l, <i>Max</i> | 30 | 100 | IS 3025 (Part 46) | — |
| xiv) | Manganese (as Mn), mg/l, <i>Max</i> | 0.1 | 0.3 | IS 3025 (Part 59) | Total concentration of manganese (as Mn) and iron (as Fe) shall not exceed 0.3 mg/l |
| xv) | Mineral oil, mg/l, <i>Max</i> | 0.5 | No relaxation | Clause 6 of IS 3025 (Part 39) Infrared partition method | — |
| xvi) | Nitrate (as NO ₃), mg/l, <i>Max</i> | 45 | No relaxation | IS 3025 (Part 34) | — |
| xvii) | Phenolic compounds (as C ₆ H ₅ OH), mg/l, <i>Max</i> | 0.001 | 0.002 | IS 3025 (Part 43) | — |
| xviii) | Selenium (as Se), mg/l, <i>Max</i> | 0.01 | No relaxation | IS 3025 (Part 56) or IS 15303* | — |
| xix) | Silver (as Ag), mg/l, <i>Max</i> | 0.1 | No relaxation | Annex J of IS 13428 | — |
| xx) | Sulphate (as SO ₄) mg/l, <i>Max</i> | 200 | 400 | IS 3025 (Part 24) | May be extended to 400 provided that Magnesium does not exceed 30 |
| xxi) | Sulphide (as H ₂ S), mg/l, <i>Max</i> | 0.05 | No relaxation | IS 3025 (Part 29) | — |
| xxii) | Total alkalinity as calcium carbonate, mg/l, <i>Max</i> | 200 | 600 | IS 3025 (Part 23) | — |
| xxiii) | Total hardness (as CaCO ₃), mg/l, <i>Max</i> | 200 | 600 | IS 3025 (Part 21) | — |
| xxiv) | Zinc (as Zn), mg/l, <i>Max</i> | 5 | 15 | IS 3025 (Part 49) | — |

NOTES

1 In case of dispute, the method indicated by '*' shall be the referee method.

2 It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 3 Parameters Concerning Toxic Substances
(Foreword and Clause 4)

| Sl No. | Characteristic | Requirement (Acceptable Limit) | Permissible Limit in the Absence of Alternate Source | Method of Test, Ref to | Remarks |
|--------|--|--------------------------------|--|--|-------------------|
| (1) | (2) | (3) | (4) | (5) | (6) |
| i) | Cadmium (as Cd), mg/l, <i>Max</i> | 0.003 | No relaxation | IS 3025 (Part 41) | — |
| ii) | Cyanide (as CN), mg/l, <i>Max</i> | 0.05 | No relaxation | IS 3025 (Part 27) | — |
| iii) | Lead (as Pb), mg/l, <i>Max</i> | 0.01 | No relaxation | IS 3025 (Part 47) | — |
| iv) | Mercury (as Hg), mg/l, <i>Max</i> | 0.001 | No relaxation | IS 3025 (Part 48)/ Mercury analyser | — |
| v) | Molybdenum (as Mo), mg/l, <i>Max</i> | 0.07 | No relaxation | IS 3025 (Part 2) | — |
| vi) | Nickel (as Ni), mg/l, <i>Max</i> | 0.02 | No relaxation | IS 3025 (Part 54) | — |
| vii) | Pesticides, µg/l, <i>Max</i> | See Table 5 | No relaxation | See Table 5 | — |
| viii) | Polychlorinated biphenyls, mg/l, <i>Max</i> | 0.000 5 | No relaxation | ASTM 5175* | — |
| ix) | Polynuclear aromatic hydrocarbons (as PAH), mg/l, <i>Max</i> | 0.000 1 | No relaxation | APHA 6440 | or APHA 6630 — |
| x) | Total arsenic (as As), mg/l, <i>Max</i> | 0.01 | 0.05 | IS 3025 (Part 37) | — |
| xi) | Total chromium (as Cr), mg/l, <i>Max</i> | 0.05 | No relaxation | IS 3025 (Part 52) | — |
| xii) | Trihalomethanes: | | | | |
| a) | Bromoform, mg/l, <i>Max</i> | 0.1 | No relaxation | ASTM D 3973-85* or APHA 6232 | — |
| b) | Dibromochloromethane, mg/l, <i>Max</i> | 0.1 | No relaxation | ASTM D 3973-85* or APHA 6232 | — |
| c) | Bromodichloromethane, mg/l, <i>Max</i> | 0.06 | No relaxation | ASTM D 3973-85* or APHA 6232 | — |
| d) | Chloroform, mg/l, <i>Max</i> | 0.2 | No relaxation | ASTM D 3973-85* or APHA 6232 | — |

NOTES

1 In case of dispute, the method indicated by '*' shall be the referee method.

2 It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 4 Parameters Concerning Radioactive Substances
(Foreword and Clause 4)

| Sl No. | Characteristic | Requirement (Acceptable Limit) | Permissible Limit in the Absence of Alternate Source | Method of Test, Ref to Part of IS 14194 | Remarks |
|--------|---------------------------------|--------------------------------|--|---|---------|
| (1) | (2) | (3) | (4) | (5) | (6) |
| i) | Radioactive materials: | | | | |
| a) | Alpha emitters Bq/l, <i>Max</i> | 0.1 | No relaxation | Part 2 | — |
| b) | Beta emitters Bq/l, <i>Max</i> | 1.0 | No relaxation | Part 1 | — |

NOTE — It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under 'acceptable' render the water not suitable, but still may be tolerated in the absence of an alternative source but up to the limits indicated under 'permissible limit in the absence of alternate source' in col 4, above which the sources will have to be rejected.

Table 5 Pesticide Residues Limits and Test Method
(Foreword and Table 3)

| Sl No. | Pesticide | Limit µg/l | Method of Test, Ref to | |
|--------|--|---------------|------------------------|------------------|
| | | | USEPA (4) | AOAC/ ISO (5) |
| (1) | (2) | (3) | | |
| i) | Alachlor | 20 | 525.2, 507 | — |
| ii) | Atrazine | 2 | 525.2, 8141 A | — |
| iii) | Aldrin/ Dieldrin | 0.03 | 508 | — |
| iv) | Alpha HCH | 0.01 | 508 | — |
| v) | Beta HCH | 0.04 | 508 | — |
| vi) | Butachlor | 125 | 525.2, 8141 A | — |
| vii) | Chlorpyrifos | 30 | 525.2, 8141 A | — |
| viii) | Delta HCH | 0.04 | 508 | — |
| ix) | 2,4- Dichlorophenoxyacetic acid | 30 | 515.1 | — |
| x) | DDT (<i>o, p</i> and <i>p, p</i> – Isomers of DDT, DDE and DDD) | 1 | 508 | AOAC 990.06 |
| xi) | Endosulfan (alpha, beta, and sulphate) | 0.4 | 508 | AOAC 990.06 |
| xii) | Ethion | 3 | 1657 A | — |
| xiii) | Gamma — HCH (Lindane) | 2 | 508 | AOAC 990.06 |
| xiv) | Isoproturon | 9 | 532 | — |
| xv) | Malathion | 190 | 8141 A | — |
| xvi) | Methyl parathion | 0.3 | 8141 A | ISO 10695 |
| xvii) | Monocrotophos | 1 | 8141 A | — |
| xviii) | Phorate | 2 | 8141 A | — |

NOTE — Test methods are for guidance and reference for testing laboratory. In case of two methods, USEPA method shall be the reference method.

Table 6 Bacteriological Quality of Drinking Water¹⁾
(Clause 4.1.1)

| Sl No. | Organisms | Requirements |
|--------|--|--|
| (1) | (2) | (3) |
| i) | <i>All water intended for drinking:</i> | |
| a) | <i>E. coli</i> or thermotolerant coliform bacteria ^{2), 3)} | Shall not be detectable in any 100 ml sample |
| ii) | <i>Treated water entering the distribution system:</i> | |
| a) | <i>E. coli</i> or thermotolerant coliform bacteria ²⁾ | Shall not be detectable in any 100 ml sample |
| b) | Total coliform bacteria | Shall not be detectable in any 100 ml sample |
| iii) | <i>Treated water in the distribution system:</i> | |
| a) | <i>E. coli</i> or thermotolerant coliform bacteria | Shall not be detectable in any 100 ml sample |
| b) | Total coliform bacteria | Shall not be detectable in any 100 ml sample |

¹⁾Immediate investigative action shall be taken if either *E.coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause shall be determined by immediate further investigation.

²⁾Although, *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests shall be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

³⁾It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for progressive improvement of water supplies.

system including consumers' premises, should be free from virus.

4.2.2 None of the generally accepted sewage treatment methods yield virus-free effluent. Although a number of investigators have found activated sludge treatment to be superior to trickling filters from this point of view, it seems possible that chemical precipitation methods will prove to be the most effective.

4.2.3 Virus can be isolated from raw water and from springs, enterovirus, reovirus, and adenovirus have been found in water, the first named being the most resistant to chlorination. If enterovirus are absent from chlorinated water, it can be assumed that the water is safe to drink. Some uncertainty still remains about the virus of infectious hepatitis, since it has not so far been isolated but in view of the morphology and resistance of enterovirus it is likely that, if they have been inactivated hepatitis virus will have been inactivated also.

4.2.4 An exponential relationship exists between the rate of virus inactivation and the redox potential. A redox potential of 650 mV (measured between platinum and calomel electrodes) will cause almost instantaneous inactivation of even high concentrations of virus. Such a potential can be obtained with even a low concentration of free chlorine, but only with an extremely high concentration of combined chlorine. This oxidative inactivation may be achieved with a number of other oxidants also, for example, iodine, ozone and potassium permanganate, but the effect of the oxidants will always be counteracted, if reducing components, which are mainly organic, are present. As a consequence, the sensitivity of virus towards disinfectants will depend on the *milieu* just as much as on the particular disinfectant used.

4.2.5 Viruses are generally resistant to disinfectants as well as get protected on account of presence of particulate and organic matter in water. Because the difference between the resistance of coliform organisms and of virus to disinfection by oxidants increases with increasing concentration of reducing components, for example, organic matter, it cannot be assumed that the absence of available coliform organisms implies freedom from active virus under circumstances where a free chlorine residual cannot be maintained. Sedimentation and slow sand filtration in themselves may contribute to the removal of virus from water.

4.2.6 In practice, >0.5 mg/l of free chlorine for 1 h is sufficient to inactivate virus, even in water that was originally polluted provided the water is free from particulates and organic matter.

4.2.7 MS2 phage are indicator of viral contamination in drinking water. MS2 phage shall be absent in 1 litre of water when tested in accordance with USEPA method 1602. If MS2 phage are detected in the drinking water, virological examination shall be done by the Polymerase Chain Reaction (PCR) method for virological examination as given in Annex B. USEPA method in Manual of Method for Virology Chapter 16, June 2001 shall be the alternate method. If viruses are detected, the cause shall be determined by immediate further investigation.

4.3 Biological Requirements

4.3.1 Ideally, all samples taken including consumers premises should be free from biological organisms. Biological examination is of value in determining the causes of objectionable tastes and odours in water and controlling remedial treatments, in helping to interpret the results of various chemical analysis, and in explaining the causes of clogging in distribution pipes and filters. In some instances, it may be of use in demonstrating that water from one source has been mixed with that from another.

4.3.2 The biological qualities of water are of greater importance when the supply has not undergone the conventional flocculation and filtration processes, since increased growth of methane-utilizing bacteria on biological slimes in pipes may then be expected, and the development of bryozoal growths such as *Plumatella* may cause operational difficulties.

4.3.3 Some of the animalcules found in water mains may be free-living in the water, but others such as *Dreissena* and *Asellus* are more or less firmly attached to the inside of the mains. Although these animalcules are not themselves pathogenic, they may harbour pathogenic organisms or virus in their intestines, thus protecting these pathogens from destruction by chlorine.

4.3.4 Chlorination, at the dosages normally employed in waterworks, is ineffective against certain parasites, including amoebic cysts; they can be excluded only by effective filtration or by higher chlorine doses than can be tolerated without subsequent dechlorination. *Amoebiasis* can be conveyed by water completely free from enteric bacteria; microscopic examination after concentration is, therefore, the only safe method of identification.

4.3.5 Strict precautions against back-syphonage and cross-connections are required, if amoebic cysts are found in a distribution system containing tested water.

4.3.6 The *cercariae of schistosomiasis* can be detected by similar microscopic examination, but there is, in

any case, no evidence to suggest that this disease is normally spread through piped water supplies.

4.3.7 The cyclops vector of the embryos of *Dracunculus medinensis* which causes dracontiasis or Guinea-worm disease can be found in open wells in a number of tropical areas. They are identifiable by microscopic examination. Such well supplies are frequently used untreated, but the parasite can be relatively easily excluded by simple physical improvements in the form of curbs, drainage, and apron surrounds and other measures which prevent physical contact with the water source.

4.3.8 Cryptosporidium shall be absent in 10 liter of water when tested in accordance with USEPA method 1622 or USEPA method 1623* or ISO 15553 : 2006.

4.3.9 Giardia shall be absent in 10 liter of water when tested in accordance with USEPA method 1623* or ISO 15553 : 2006.

4.3.10 The drinking water shall be free from microscopic organisms such as algae, zooplanktons, flagellates, parasites and toxin producing organisms. An illustrative (and not exhaustive) list is given in Annex C for guidance.

NOTE — In case of dispute, the method indicated by '*' in **4.3.8** and **4.3.9** shall be referee method.

5 SAMPLING

Representative samples of water shall be drawn as prescribed in IS 1622 and IS 3025 (Part 1).

ANNEX A

(Clause 2)

LIST OF REFERRED INDIAN STANDARDS

| IS No. | Title | IS No. | Title |
|------------------|---|------------------|---|
| 1622 : 1981 | Methods of sampling and microbiological examination of water (<i>first revision</i>) | (Part 41) : 1992 | Cadmium (<i>first revision</i>) |
| | | (Part 42) : 1992 | Copper (<i>first revision</i>) |
| 3025 | Methods of sampling and test (physical and chemical) for water and waste water: | (Part 43) : 1992 | Phenols (<i>first revision</i>) |
| (Part 1) : 1987 | Sampling (<i>first revision</i>) | (Part 46) : 1994 | Magnesium |
| (Part 2) : 2002 | Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy | (Part 47) : 1994 | Lead |
| (Part 4) : 1983 | Colour (<i>first revision</i>) | (Part 48) : 1994 | Mercury |
| (Part 5) : 1983 | Odour (<i>first revision</i>) | (Part 49) : 1994 | Zinc |
| (Part 7) : 1984 | Taste threshold (<i>first revision</i>) | (Part 52) : 2003 | Chromium |
| (Part 8) : 1984 | Tasting rate (<i>first revision</i>) | (Part 53) : 2003 | Iron |
| (Part 10) : 1984 | Turbidity (<i>first revision</i>) | (Part 54) : 2003 | Nickel |
| (Part 11) : 1983 | pH value (<i>first revision</i>) | (Part 55) : 2003 | Aluminium |
| (Part 16) : 1984 | Filterable residue (total dissolved solids) (<i>first revision</i>) | (Part 56) : 2003 | Selenium |
| (Part 21) : 1983 | Total hardness (<i>first revision</i>) | (Part 57) : 2005 | Boron |
| (Part 23) : 1983 | Alkalinity (<i>first revision</i>) | (Part 59) : 2006 | Manganese |
| (Part 24) : 1986 | Sulphates (<i>first revision</i>) | (Part 60) : 2008 | Fluoride |
| (Part 26) : 1986 | Chlorine residual (<i>first revision</i>) | 13428 : 2003 | Packaged natural mineral water — Specification (<i>first revision</i>) |
| (Part 27) : 1986 | Cyanide (<i>first revision</i>) | 14194 | Radionuclides in environmental samples — Method of estimation: |
| (Part 29) : 1986 | Sulphide (<i>first revision</i>) | (Part 1) : 1994 | Gross beta activity measurement |
| (Part 32) : 1988 | Chloride (<i>first revision</i>) | (Part 2) : 1994 | Gross alpha activity measurement |
| (Part 34) : 1988 | Nitrogen (<i>first revision</i>) | 15302 : 2002 | Determination of aluminium and barium in water by direct nitrous oxide-acetylene flame atomic absorption spectrometry |
| (Part 37) : 1988 | Arsenic (<i>first revision</i>) | 15303 : 2002 | Determination of antimony, iron and selenium in water by electrothermal atomic absorption spectrometry |
| (Part 39) : 1989 | Oil and grease | | |
| (Part 40) : 1991 | Calcium | | |

ANNEX B

(Clause 4.2.7)

POLYMERASE CHAIN REACTION (PCR) METHOD

B-1 GENERAL

The method involves the concentration of viruses from 100 litre of drinking water to 1 ml by membrane filter technique. The concentrate is subjected to amplification using polymerase chain reaction (PCR) and primers based on highly conserved regions of viral genomes. This method can detect as low as 10 genome copies. Stringent precautions are needed to avoid contamination with amplified DNA products leading to false positive reactions. Detection of hepatitis A virus (HAV) RNA and enterovirus (EV) RNA is considered as an indication of presence of viruses in water. Steps involved include concentration of water, RNA extraction, complementary DNA (cDNA) synthesis and PCR.

B-2 CONCENTRATION OF DRINKING WATER

B-2.1 Apparatus

B-2.1.1 Pressure Pump

B-2.1.2 Membrane Filter Assembly with 144 mm Diameter with Tripod Stand

B-2.1.3 Pressure Vessel (50 litre capacity) with Pressure Gauge

B-2.1.4 Inter-connecting Pressure Tubes

B-2.2 Reagents

Autoclaved double distilled water shall be used for the preparation of reagents/buffers in this study.

B-2.2.1 Aluminium Chloride

B-2.2.2 HCl/NaOH Urea (Extra Pure)

B-2.2.3 Disodium Hydrogen Phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) — 0.2 M, filter sterilized.

B-2.2.4 Sodium Dihydrogen Phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) — 0.2 M, filter sterilized.

B-2.2.5 Citric Acid — 0.1 M, filter sterilized.

B-2.2.6 L-Arginine — 0.5 M, filter sterilized.

B-2.2.7 Urea-Arginine Phosphate Buffer (U-APB) — Mix 4.5 g of urea with 2 ml of 0.2 M NaH_2PO_4 and 2 ml of 0.5 M L - Arginine and make up the volume to 50 ml with sterile distilled water. The pH of the eluent shall be 9.0.

B-2.2.8 Magnesium Chloride (MgCl_2) — 1 M.

B-2.2.9 McII Vaines Buffer (pH 5.0) — Mix 9.7 ml of

0.1 M citric acid with 10.3 ml of 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ under sterile conditions.

B-2.3 Procedure

Filter 100 litre of drinking water sample through membrane filter assembly using either positively charged membrane of 144 mm diameter or 0.22 micron diameter pore size nitrocellulose membrane. For positively charged membrane the test water pH need not be adjusted. But for the 0.22 micron nitrocellulose membrane adjust the pH to 3.5 after adding the aluminium chloride as a coagulant to a final concentration of 0.000 5 M.

At lower pH pass the water through the membrane. The flow rate shall be 40 litre/h approximately. After the completion of the filtration, elute the adsorbed particles using 100 ml of urea-arginine phosphate buffer (U-APB). Precipitate the suspended particles using 1 ml of magnesium chloride (1 M). Dissolve the resultant precipitate centrifuged out of the sample in 800-1.0 ml of McII vaines buffer. The processed sample can be stored at refrigerator until required.

B-3 RNA EXTRACTION

B-3.1 Apparatus

B-3.1.1 Cooling Centrifuge

B-3.1.2 Deep Freezer (-20°C)

B-3.1.3 Vortex Mixer

B-3.1.4 Pipette Man

B-3.2 Reagents

B-3.2.1 Cetyl Trimethyl Ammonium Bromide (CTAB) Buffer

| | | |
|-------------------------------|---|-----------|
| CTAB | : | 1 percent |
| Sodium Dodecyl Sulphate (SDS) | : | 1 percent |
| EDTA | : | 20 mM |
| Sodium Chloride | : | 1 M |

B-3.2.2 Phenol, Chloroform and Isoamylalcohol in the ratio of 25:24:1 (PCI)

B-3.2.3 Ethanol

B-3.2.4 TE Buffer (pH 8.0)

| | | |
|-----------|---|-------|
| Tris base | : | 1 M |
| EDTA | : | 0.5 M |

B-3.2.5 Sodium Acetate — 3 M.

B-3.3 Procedure

Treat 300 µl of concentrated water sample with equal volume of CTAB and 1/10th volume of PCI. Vortex and centrifuge at 5 000 × g for 30 min at 4°C. Add 1/10th volume of 3 M sodium acetate and double the volume of cold ethanol to the aqueous layer. Keep the mixture at either at -20°C for overnight or in liquid nitrogen for 2-5 min. Centrifuge at 10 000 × g, for 30 min at 4°C. Discard the supernatant and air dry the pellet and dissolve it in 20 µl TE buffer.

B-4 COMPLEMENTARY DNA (c DNA) SYNTHESIS**B-4.1 Apparatus****B-4.1.1 PCR Machine****B-4.1.2 Deep Freezer (-20°C)****B-4.2 Reagents****B-4.2.1 cDNA Synthesis Kit****B-4.3 Procedure**

Suspend the extracted RNA in 20 µl of cDNA reaction mixture, which consists of 4 µl of 5X reverse transcriptase reaction buffer [250 mM TRIS-HCl (pH 8.5), 40 mM KCl, 150 mM MgCl₂, 5 mM dithiothreitol (DTT)], 0.5 µl of 10 mM deoxynucleotide phosphate (dNTP), 2 µl of hexa nucleotide mixture, 1 µl of 25 U of Maloney Murine Leukaemia Virus (M-MuLV) reverse transcriptase, 0.5 µl of 20 U of human placental RNase inhibitor. Heat the reaction mixture to 95°C for 5 min and rapidly chill on ice, this is followed by the addition of 1 µl (25 U/µl) of M-MuLV reverse transcriptase. Incubate the reaction mixture as given by the manufacturer of the kit and quickly chill the reaction tube on ice.

B-5 PCR AMPLIFICATION**B-5.1 Apparatus****B-5.1.1 PCR Machine****B-5.1.2 Deep Freezer (-20°C)****B-5.1.3 Micropipette****B-5.2 Reagents****B-5.2.1 Primers for EV and HAV**

EV sense primer, 5' — TCC TCC GGC CCC
TGA ATG CG — 3'
antisense primer, 5' — ATT GTC ACC
ATA AGC AGC CA — 3'
HAV sense primer, 5' — GTTTT GCTCC
TCTTT ATCAT GCTAT G-3'

antisense primer, 5' — GGAAA TGTCT
CAGGT ACTTT CTTTG-3'

B-5.2.2 PCR Master Mix**B-5.2.3 Mineral Oil****B-5.3 Procedure****B-5.3.1 PCR Amplification for Hepatitis A Virus (HAV)**

In 5 µl of cDNA, add 95 µl of a PCR Master Mix (10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.01 percent gelatin (1× PCR buffer), 200 µM of each dNTP, 1.5 U of *Thermus aquaticus* polymerase). Add 25 pico moles of sense and antisense oligonucleotide primers of HAV and overlay with mineral oil. Appropriate positive and negative controls shall be included with each run. Set the following reaction at thermo cycler:

| | |
|------------------------------------|-------------|
| Denaturation at 94°C for 2 min | } 35 cycles |
| Denaturation for 1.0 min at 94°C | |
| Annealing for 1.0 min at 57°C | |
| Extension for 1.3 min at 72°C | |
| Final extension at 72°C for 7 min. | |

B-5.3.2 PCR Amplification for Enterovirus (EV)

In 5 µl of cDNA, add 95 µl of a PCR Master Mix (10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.01 percent gelatin (1X PCR buffer), 200 µM of each dNTP, 1.5 U of *Thermus aquaticus* polymerase). Add 25 pico moles of sense and antisense oligonucleotide primers of EV and overlay with mineral oil. Appropriate positive and negative controls shall be included with each run. Set the following reaction at thermo cycler:

| | |
|------------------------------------|-------------|
| Denaturation at 94°C for 2 min | } 35 cycles |
| Denaturation for 1.0 min at 94°C | |
| Annealing for 1.0 min at 42°C | |
| Extension for 2.0 min at 72°C | |
| Final extension at 72°C for 7 min. | |

B-6 AGAROSE GEL ELECTROPHORESIS**B-6.1 Apparatus****B-6.1.1 Micropipette****B-6.1.2 Electrophoresis Apparatus****B-6.1.3 Gel Documentation System****B-6.2 Reagents****B-6.2.1 Running Buffer — 50X TAE buffer**

Tris base/Tris buffer : 121.00 g

Glacial acetic acid : 28.55 ml
 0.5 M EDTA : 50 .00 ml
 Distilled water : 300.45 ml
 (autoclaved)

Make the final volume upto 1 000 ml with deionised distilled water, sterilize and store at 4°C. The final concentration for the preparation of agarose gel and to run the gel shall be 1X.

B-6.2.2 Tracking Dye — 6X bromophenol blue.

B-6.2.3 Ethidium Bromide — 0.5 µg/ml.

B-6.3 Procedure

Run the PCR amplified product of EV and HAV on 1.5 percent agarose gel using 1X TAE buffer. Load 10 µl of amplified product after mixing it with 1 µl 10X loading dye. Run the molecular weight marker along with the samples. Run the electrophoresis at 100 V for 30 min. Stain the gel with ethidium bromide (0.5 µl/ml) for 20 min. Wash it with distilled water and view under UV transilluminator and photograph the gel to analyse the band pattern. EV gives the band as 155 base pair and the HAV gives band as 225 base pair.

ANNEX C (Clause 4.3.10)

ILLUSTRATIVE LIST OF MICROSCOPIC ORGANISMS PRESENT IN WATER

| Sl No. | Classification of Microscopic Organism | Group and Name of the Organism | Habitat | Effect of the Organisms and Significance |
|--------|--|--|------------------------------------|--|
| (1) | (2) | (3) | (4) | (5) |
| i) | Algae | a) Chlorophyceae: | | |
| | | 1) <i>Species of</i> Coelastrum, Gomphospherium, Micractinium, Mougeotia, Oocystis, Euastrum, Scenedesmus, Actinastrum, Gonium, Eudorina Pandorina, Pediastrum, Zygnema, Chlamydomonas, Careteria, Chlorella, Chroococcus, Spirogyra, Tetradron, Chlorogonium, Stigeoclonium | Polluted water, impounded sources | Impart colouration |
| | | 2) <i>Species of</i> Pandorina, Volvox, Gomphospherium, Staurastrum, Hydrodictyon, Nitella | Polluted waters | Produce taste and odour |
| | | 3) <i>Species of</i> Rhizoclonium, Cladotrix, Ankistrodesmus, Ulothrix, Micrasterias, Chromulina | Clean water | Indicate clean condition |
| | | 4) <i>Species of</i> Chlorella, Tribonema, Clostrium, Spirogyra, Palmella | Polluted waters, impounded sources | Clog filters and create impounded difficulties |
| | | b) Cyanophyceae: | | |
| | | 1) <i>Species of</i> Anacystis and Cylandrospermum | Polluted waters | Cause water bloom and impart colour |
| | | 2) <i>Species of</i> Anabena, Phormidium, Lyngbya, Arthrospira, Oscillatoria | Polluted waters | Impart colour |
| | | 3) <i>Species of</i> Anabena, Anacystis, Aphanizomenon | Polluted waters, impounded sources | Produce taste and odour |
| | | 4) <i>Species of</i> Anacystis, Anabena, Coelospherium, Cleotrichina, Aphanizomenon | Polluted waters | Toxin producing |
| | | 5) <i>Species of</i> Anacystis, Rivularia, Oscillatoria, Anabena | Polluted waters | Clog filters |

| <i>Sl No.</i> | <i>Classification of Microscopic Organism</i> | <i>Group and Name of the Organism</i> | <i>Habitat</i> | <i>Effect of the Organisms and Significance</i> |
|---------------|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) |
| | | 6) <i>Species of Rivularia</i> | Calcareous waters and also rocks | Bores rocks and calcareous strata and causes matted growth |
| | | 7) <i>Species of Lemanea</i> | Agmenellum, Microcoleus, Clean waters | Indicators of purification |
| | | c) Diatoms (Bacillareophyceae): | | |
| | | 1) <i>Species of Stauroneis</i> | Fragillaria, Stephanodiscus, — | Cause discoloration |
| | | 2) <i>Species of Asterionella</i> | Tabellaria | Hill streams high altitude, torrential and temperate waters |
| | | 3) <i>Species of Synedra</i> | and Fragillavia | Polluted waters |
| | | 4) <i>Species of Nitzchia</i> | Gomphonema | Moderately polluted waters |
| | | 5) <i>Species of Cymbela</i> | Synedra, Melosira, Rivers and streams impounded sources | Clog filters and cause operational difficulties |
| | | 6) <i>Species of Pinnularia</i> | Surinella, Clean waters | Indicators of purification |
| | | d) Xanthophyceae: | | |
| | | <i>Species of Botryococcus</i> | Hill streams, high altitude and temperate waters | Produces coloration |
| ii) | Zooplankton | a) Protozoa: | | |
| | | 1) Amoeba, Giardia, Lamblia, Arcella, Diffugia, Actinophrys | Polluted waters | Pollution indicators |
| | | 2) Endamoeba, Histolytica | Sewage and activated sludge | Parasitic and pathogenic |
| | | b) Ciliates: | | |
| | | Paramoecium, Vorticella, Carchesium, Stentor, Colpidium, Coleps, Euplotes, Colopoda, Bodo | Highly polluted waters, sewage and activated sludge | Bacteria eaters |
| | | c) Crustacea: | | |
| | | 1) Bosmina, Daphnia | Stagnant polluted waters | Indicators of pollution |
| | | 2) Cyclops | Step wells in tropical climate | Carrier host of guinea worm |
| iii) | Rotifers | a) Rotifers: | | |
| | | Anurea, Rotaria, Philodina | Polluted and Algae laden waters | Feed on algae |
| | | b) Flagellates: | | |
| | | 1) Ceratium, Glenodinium, Dinobryon | Peridinium | Rocky strata, iron bearing and acidic waters |
| | | 2) Euglena, Phacus | Polluted waters | Impart colour |

| <i>Sl No.</i> | <i>Classification of Microscopic Organism</i> | <i>Group and Name of the Organism</i> | <i>Habitat</i> | <i>Effect of the Organisms and Significance</i> |
|---------------|---|---------------------------------------|---|---|
| (1) | (2) | (3) | (4) | (5) |
| iv) | Miscellaneous Organisms | a) Sponges, Hydra | Fresh water | Clog filters and affect purification systems |
| | | b) Tubifex, Eristalls, Chironomids | Highly polluted waters, sewage and activated sludge and bottom deposits | Clog filters and render water unaesthetic |
| | | c) Plumatella | Polluted waters | Produces biological slimes and causes filter operational difficulties |
| | | c) Dreissena, Asellus | Polluted waters | Harbour pathogenic organisms |

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