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Draft Indian Standard

DRINKING WATER – SPECIFICATION

(*Second Revision* of IS 10500)

ICS No. 13.060.20

BUREAU OF INDIAN STANDARDS
MANAK BHAWAN, 9 BAHADUR SHAH ZAFAR MARG
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Date **Price Group**

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FOREWORD

(Formal clauses will be added later.)

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 prepared with the objective of assessing the quality of water resources, and to check the effectiveness of water treatment and supply by the concerned authorities.

While preparing the standard, Committee took due note of the limited testing facilities available in the country. The standard therefore categorized various characteristics as essential or desirable.

During VII Five-Year Plan, 55 mini mission districts were identified with a view to meet supply of water to all the problem villages. The VIII Five-Year Plan intended to provide safe drinking water to the rural masses. It also proposed to ensure supply of desired quality and required quantity of drinking water.

The first revision was undertaken to take into account the uptodate 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.

In the formulation of the first revision, assistance was derived from the following publications:

a) International Standards for Drinking Water issued by World Health Organization, 1984 Geneva;

b) Manual of Standards of Quality for Drinking Water Supplies. Indian Council of Medical Research, 1971, New Delhi; and

c) Manual on Water Supply and Treatment (third *revision),* Ministry of Urban Development, 1989, New Delhi

The tenth five year Plan document of India (2002-2007) has emphasized protection of the environment and safeguarding of health through the integrated management of water resources and liquid and solid waste.

Need was felt to upgrade the requirements of the standard and align with the internationally available specifications on Drinking water. The second revision was undertaken for this purpose. In the second revision the following were considered:

i) EU Directives relating to the quality of water intended for human consumption (80/778/EEC) and Council Directive 98/83/EC.

ii) USEPA standard - National primary drinking water standard. EPA 816-F-02-013 dated July, 2002

iii) WHO Guidelines for Drinking Water Quality. 3rd Edition Vol. 2 Health Criteria and other supporting information.

iv) Manual on Water supply and treatment, third edition - revised and updated May 1999, Ministry of Urban Development, New Delhi

The standard mentions the acceptable limit and indicates its background. It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under Acceptable render the water not acceptable, but still may be tolerated in the absence of an alternative source but upto the limits indicated under permissible limit in the absence of alternate source in col (5) Table 1 to 4 of the Specification, above which the sources will have to be rejected.

Pesticide residues limits and test methods given in Table 5 of the standard 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 the second revision, test method for virological examination has been given in specification.

Routine surveillance of drinking water supplies must be carried out by the relevant authorities to understand the risk of specific pathogens and to define proper control procedures. Precautions/care should be taken to prevent contamination of drinking water from chlorine resistant parasites such as cryptosporidium species and giardia.

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DRINKING WATER – SPECIFICATION
(*Second Revision* of IS 10500)

1 SCOPE

The standard prescribes the requirements, test methods and sampling procedure for ascertaining the suitability of water for drinking purpose.

2 REFERENCES

The Indian Standards listed in Annex A contain provisions which through reference in this text, constitute provisions of this Indian Standard. At the time of publication, the editions indicated were valid. All standards are subject to revisions 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 supplied by pipes or any other means for human consumption by any supplier.

4 REQUIREMENTS

Drinking water shall comply with the requirements given in Table 1, Table 2, Table 3 and Table 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 (**4.1**) Virological requirements (**4.2**) and Biological requirements (**4.3**).

4.1 Bacteriological Requirements

4.1.1 *Water in Distribution System*

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

4.1.1.1 If any coliform organisms are found the minimum action required is immediate resampling and examination. Measures should at once be taken to discover the source of contamination and remove the source of the pollution.

Table 1 Organoleptic and Physical parameters
(*Clause* **4)**

Sl. No.	Substance or characteristic	Requirement (Acceptable Limit)	Undesirable effect outside the acceptable limit	Permissible limit in the absence of alternate source	Method of test (Ref to IS)	Remarks
i)	Colour, Hazen units, *Max*	5	Above 5 consumer acceptance decreases	15	3025 (Part 4)	Extended to 15 only if toxic substances are not suspected in absence of alternate sources.
ii)	Odour	Agreeable	-	Agreeable	3025 (Part 5)	a)Test cold and when heated b) Test at several dilutions.
iii)	Taste	Agreeable	-	Agreeable	3025 (Part 7 and 8)	Test to be conducted only after safety has been established.
iv)	Turbidity, NTU, Max	1	Above 5 consumer acceptance decreases	5	3025 (Part 10)	-
v)	Dissolved solids, mg/l, Max	500	Beyond this palatability decreases and may cause gastrointestinal irritation.	2000	3025 (Part 16)	-
vi)	pH value	6.5-8.5	Beyond this range the water will affect the mucous membrane and/or water supply system.	No Relaxation	3025 (Part 11)	-
vii)	Total hardness (as CaCO3), mg/l, Max	200	Encrustation in water supply structure and adverse effects on domestic use.	600	3025 (Part 21)	-

NOTE 1: It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under Acceptable render the water not acceptable, but still may be tolerated in the absence of an alternative source but upto the limits indicated under permissible limit in the absence of alternate source in col (5), above which the sources will have to be rejected.

Table 2 General Parameters concerning substances undesirable in excessive amounts
(*Clause* 4)

Sl. No.	Substance or characteristic	Requirement (Acceptable Limit)	Undesirable effect outside the acceptable limit	Permissible limit in the absence of alternate source	Method of test (Ref to IS)	Remarks
i)	Iron (as Fe), mg/l, *Max*	0.3	Beyond this limit taste/appearance are affected, has adverse effect on domestic uses and water supply structures, and promotes iron bacteria	No relaxation	3025 (Part 53)	Total concentration of Manganese (as Mn) and Iron (as Fe) shall not exceed 0.3 mg/l
ii)	Aluminium (as Al), mg/l, *Max*	0.03	Cumulative effect is reported to cause dementia.	0.2	3025 (Part 55)	-
iii)	Copper (as Cu), mg/l, *Max*	0.05	Astringent taste, discoloration and corrosion of pipes, fitting and utensils will be caused beyond this.	1.5	3025 (Part 42)	-
iv)	Manganese (as Mn), mg/l, *Max*	0.1	Beyond this limit taste/appearance are affected, has adverse effect on domestic uses and water supply structures.	0.3	IS 3025 (Part 59)	Total concentration of Manganese (as Mn) and Iron (as Fe) shall not exceed 0.3 mg/l
v)	Zinc (as Zn), mg/l, *Max*	5	Beyond this limit it can cause astringent taste and an opalescence in water.	15	3025 (Part 49)	-
vi)	Magnesium (as Mg), mg/l, *Max*	30	Encrustation in water supply structure and adverse effects on domestic use.	No Relaxation	3025 (Part 46)	-
vii)	Barium (as Ba), mg/l, *Max*	0.7	May lead to cardiovascular problem.	No relaxation	Annex F of IS 13428*/IS 15302	-

viii)	Calcium (as Ca), mg/l, *Max*	75	Encrustation in water supply structure and adverse effects on domestic use.	200	3025 (Part 40)	-
ix)	Silver (as Ag), mg/l, *Max*	0.1	-	No relaxation	Annex J 0f IS 13428	-
x)	Selenium (as Se), mg/l, *Max*	0.01	Beyond this, the water becomes toxic.	No relaxation	3025 (Part 56) or IS 15303*	-
xi)	Molybdenum (as Mo), mg/l, *Max*	0.07	Beyond this it may cause osteoporosis/ bone disorders.	No relaxation	3025 (Part 2;2002)/ ISO 11885: 1996	-
xii)	Boron (as B), mg/l, *Max*	0.5	-	1.0	IS 3025 (Part 57)	-
xiii)	Nitrate (as NO3) mg/l, *Max*	45	Beyond this methaemoglobinamia takes place/may be indicative of pollution	No relaxation	3025 (Part 34)	-
xiv)	Sulphate (as SO4) mg/l, *Max*	200	Beyond this causes gastro intestinal irritation when magnesium or sodium is present.	400	3025 (Part 24)	May be extended to 400 provided that Mg does not exceed 30
xv)	Sulphide (as H2S), mg/l, *Max*	Below detectable limit	Beyond this it may cause objectionable taste and odour	No relaxation	3025 (Part 29)	-
xvi)	Fluoride (as F) mg/l, *Max*	1.0	Fluoride may be kept as low as possible. High fluoride may cause fluorosis.	1.5	IS 3025 (Part 60)	-
xvii)	Chlorides (as Cl) mg/l, *Max*	250	Beyond this limit taste corrosion and palatability are affected.	1000	3025 (Part 32)	-
xviii)	Ammonia (as total ammonia-N), mg/l, *Max*	0.5	Toxicological effect about 200 mg per kg of body weight.	No relaxation	IS 3025 (Part 34)	-

xix)	Chloramines (as Cl_2), mg/l, *Max*	0.2	Eyes, nose irritation, anaemia, stomach discomfort	No relaxation	IS 3025 (Part 26) or APHA 4500-Cl G.	-
xx)	Residual, Free chlorine, mg/l, *Min*	0.2	-	1	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.
xxi)	Total Alkalinity as calcium carbonate, mg/l, *Max*	200	Beyond this limit taste becomes unpleasant.	600	3025 (Part 23)	-
xxii)	Phenolic compounds (as C6H5OH) mg/l, *Max*	0.001	Beyond this may cause objectionable taste and odour.	0.002	3025 (Part 43)	-
xxiii)	Mineral Oil, mg/l, *Max*	Below detectable limit	Beyond this limit undesirable taste and odour after chlorination take place.	No relaxation	IS 3025 (Part 39) Infrared partition method	-
xxiv)	Anionic detergents (as MBAS) mg/l, Mineral Oil, mg/l, *Max*	0.2	Beyond this limit it can cause a light froth in water.	1.0	Annex K to IS 13428	-

NOTE 2: In case of dispute, the method indicated by '*' shall be the referee method.

NOTE 3: It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under Acceptable render the water not acceptable, but still may be tolerated in the absence of an alternative source but upto the limits indicated under permissible limit in the absence of alternate source in col (5), above which the sources will have to be rejected.

Table 3 Parameters concerning toxic substances
(Clause 4)

Sl. No.	Substance or characteristic	Requirement (Acceptable Limit)	Undesirable effect outside the acceptable limit	Permissible limit in the absence of alternate source	Method of test (Ref to IS)	Remarks
i)	Total Chromium (as Cr6+), mg/l, *Max*	0.05	May be carcinogenic above this limit	No relaxation	3025 (Part 52)	-
ii)	Total Arsenic (as As), mg/l, *Max*	0.01	Beyond this the water becomes toxic	0.05	3025 (Part 37)	-
iii))	Mercury (as Hg), mg/l, *Max*	0.001	Beyond this the water becomes toxic	No relaxation	3025 (Part 48)/ Mercury Analyser	-
iv)	Cadmium (as Cd), mg/l, *Max*	0.003	Beyond this the water becomes toxic	No relaxation	3025 (Part 41)	-
v)	Lead (as Pb), mg/l, *Max*	0.01	Beyond this the water becomes toxic	No relaxation	3025 (Part 47)	-
vi)	Nickel (as Ni), mg/l, *Max*	0.02	Beyond this it may cause allergic reaction.	No relaxation	3025 (Part 54)	-
vii)	Cyanide (as CN), mg/l, *Max*	0.05	Beyond this the water becomes toxic	No relaxation	3025 (Part 27)	-
viii)	Polynuclear Aromatic Hydrocarbons (as PAH), mg/l, *Max*	0.0001	May be carcinogenic	No relaxation	APHA 6440	-
ix)	Polyclorinated biphenyls mg/l, *Max*	0.0005	May be carcinogenic	No relaxation	ASTM 5175/ APHA 6630	-
x)	Trihalomethanes					
a)	Bromoform mg/l, *Max*	0.1	May be carcinogenic above this limit	No relaxation	ASTM D 3973-85/ APHA	-

b)	Dibromochloro methane mg/l, *Max*	0.1	May be carcinogenic above this limit	No relaxation	ASTM D 3973-85/ APHA	-
c)	Bromodichloro methane mg/l, *Max*	0.1	May be carcinogenic above this limit	No relaxation	ASTM D 3973-85/ APHA	-
d)	Chloroform mg/l, *Max*	0.1	May be carcinogenic above this limit	No relaxation	ASTM D 3973-85/ APHA	-
xi)	Pesticides mg/l, *Max*	Table 5	Toxic	No relaxation	Table 5	-

NOTE 4: It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under Acceptable render the water not acceptable, but still may be tolerated in the absence of an alternative source but upto the limits indicated under permissible limit in the absence of alternate source in col (5), above which the sources will have to be rejected.

Table 4 Parameters concerning radioactive substances
(Clause 4)

Sl. No.	Substance or characteristic	Requirement (Acceptable Limit)	Undesirable effect outside the acceptable limit	Permissible limit in the absence of alternate source	Method of test (Ref to IS)	Remarks
i)	Radioactive Materials					
a)	Alpha emitters Bq/l, *Max*	0.1	May be carcinogenic above this limit	0.1	IS 14194 (Pt. 2)	-
b)	Beta emitters Bq/l, *Max*	1.0	May be carcinogenic above this limit	1.0	IS 14194 (Pt. 1)	-

NOTE 5: It is recommended that the acceptable limit is to be implemented. Values in excess of those mentioned under Acceptable render the water not acceptable, but still may be tolerated in the absence of an alternative source but upto the limits indicated under permissible limit in the absence of alternate source in col (5), above which the sources will have to be rejected.

Table 5 Pesticide residues limits and test method

Sl. No.	**Pesticide**	**Limit µg/l**	**Test method** **USEPA** **AOAC/ ISO**
i)	DDT (o,p and p,p – Isomers of DDT, DDE and DDD)	1	508 AOAC 990.06
ii)	Gamma – HCH (Lindane)	2	508 AOAC 990.06
iii)	2,4- D	30	515.1
iv)	Isoproturon	9	532
v)	Alachor	20	525.2, 507
vi)	Atrazine	2	525.2, 8141 A

vii)	Aldrin/ Dieldrin	0.03	508
viii)	Alpha HCH	0.01	508
ix)	Beta HCH	0.04	508
x)	Delta HCH	0.04	508
xi)	Endosulfan (alpha, beta, and sulphate)	0.4	508 AOAC 990.06
xii)	Monocrotophos	1	8141 A
xiii)	Ethion	3	1657 A
xiv)	Chlorpyriphos	30	525.2, 8141 A
xv)	Phorate	2	8141 A
xvi)	Butachlor	125	525.2, 8141 A
xvii)	Methyl parathion	0.3	8141 A ISO 10695
xviii)	Malathion	190	8141 A

Table 6 Bacteriological quality of drinking water [a]
(Clause 4.1)

Organisms	Guidelines
All water intended for drinking E. coli or thermotolerant coliform bacteria [b,c]	Must not be detectable in any 100 ml sample.
Treated water entering the distribution system E. coli or thermotolerent coliform Bacteria [b] Total coliform bacteria	Must not be detectable in any 100 ml sample. Must not be detectable in any 100 ml sample.
Treated water in the distribution system E. coli or thermotolerent coliform Bacteria Total coliform bacteria [d]	Must not be detectable in any 100 ml sample. Must not be detectable in any 100 ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12 month period.

a) Immediate investigative action must 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 must be determined by immediate further investigation.

b) 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 must 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.

c) 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,

d) In the remaining five percent sample total coliform bacteria should not exceed ten per hundred ml.

4.2 Virological Examination

4.2.1 Ideally all samples taken from the distribution system including consumer's premises should be free from virus. It is theoretically possible that virus disease can be transmitted by water free from coliform organisms, but conclusive evidence, that this has occurred, is lacking.

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 like1y 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 Thus, in a water in which free chlorine is present, active virus will generally be absent if coliform organisms are absent. In contrast, 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, **2-3 *mg/l*** of free chlorine for one hour is sufficient to inactivate virus, even in water that was originally polluted.

4.2.7 MS2 phage are indicator of viral contamination in drinking water. MS2 phage shall be absent when tested in accordance with USEPA method 1602. If MS2 phage are detected in the drinking water, virological examination shall be done by the PCR method for virological examination at 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 must be determined by immediate further investigation.

4.3 Biological Examination

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 when tested in accordance with USEPA method 1622/ USEPA method 1623/ ISO 15553:2006.

4.3.9 Giardia shall be absent when tested in accordance with USEPA method 1623/ 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.

5 SAMPLING

Representative samples of water shall be drawn as prescribed in IS 1622:1981 and IS 3025(Part 1):1987.

ANNEX A
(*Clause* **2)**

LIST OF REFERRED INDIAN STANDARDS

IS No.	Title
1622:1981	Methods of sampling and microbiological examination of water (first revision)
3025 (Part 1): 1987	Methods of sampling and test (physical and chemical) for water and waste water : Part 1 Sampling (first revision)
3025 (Part 4):1983	Methods of sampling and test (physical and chemical) for water and waste water : Part 4 Colour (first revision)
3025 (Part 5):1983	Methods of sampling and test (physical and chemical) for water and waste water : Part 5 Odour (first revision)
3025 (Part 7):1984	Methods of sampling and test (physical and chemical) for water and waste water : Part 7 Taste threshold (first revision)
3025 (Part 8):1984	Methods of sampling and test (physical and chemical) for water and waste water : Part 8 Tasting rate (first revision)
3025 (Part 10):1984	Methods of sampling and test (physical and chemical) for water and waste water : Part 10 Turbidity (first revision)
3025 (Part 11):1983	Methods of sampling and test (physical and chemical) for water and waste water : Part 11 pH value (first revision)
3025 (Part 16):1984	Methods of sampling and test (physical and chemical) for water and waste water : Part 16 Filterable residue (Total Dissolved Solids) (first revision)
3025 (Part 21):1983	Methods of sampling and test (physical and chemical) for water and waste water : Part 21 Total hardness (first revision)
3025 (Part 23):1983	Methods of sampling and test (physical and chemical) for water and waste water : Part 23 Alkalinity (first revision)
3025 (Part 24):1986	Methods of sampling and test (physical and chemical) for water and waste water : Part 24 Sulphates (first revision)
3025 (Part 26):1986	Methods of sampling and test (physical and chemical) for water and waste water : Part 26 Chlorine residual (first revision)
3025 (Part 27):1986	Methods of sampling and test (physical and chemical) for water and waste water : Part 27 Cyanide (first revision)
3025 (Part 29):1986	Methods of sampling and test (physical and chemical) for water and waste water : Part 29 Sulphide (first revision)
3025 (Part 32):1988	Methods of sampling and test (physical and chemical) for water and waste water : Part 32 Chloride (first revision)
3025 (Part 34):1988	Methods of sampling and test (physical and chemical) for water and waste water : Part 34 Nitrogen (first revision)
3025 (Part 37):1988	Methods of sampling and test (physical and chemical) for water and waste water : Part 37 Arsenic (first revision)
3025 (Part 39):1989	Methods of sampling and test (physical and chemical) for water and waste water : Part 39 Oil and grease
3025 (Part 40):1991	Methods of sampling and test (physical and chemical) for water and waste water : Part 40 Calcium
3025 (Part 41):1992	Methods of sampling and test (physical and chemical) for water and waste water : Part 41 Cadmium (first revision)
3025 (Part 42):1992	Methods of sampling and test (physical and chemical) for water and waste water : Part 42 Copper (first revision)
3025 (Part 43):1992	Methods of sampling and test (physical and chemical) for water and waste water : Part 43 Phenols (first revision)

3025 (Part 46):1994	Methods of sampling and test (physical and chemical) for water and waste water : Part 46 Magnesium
3025 (Part 47):1994	Methods of sampling and test (physical and chemical) for water and waste water : Part 47 Lead
3025 (Part 48):1994	Methods of sampling and test (physical and chemical) for water and waste water : Part 48 Mercury
3025 (Part 49):1994	Methods of sampling and test (physical and chemical) for water and waste water : Part 49 Zinc
3025 (Part 52):2003	Methods of sampling and test (physical and chemical) for water and waste water : Part 52 Chromium
3025 (Part 53):2003	Methods of sampling and test (physical and chemical) for water and waste water : Part 53 Iron
3025 (Part 54):2003	Methods of sampling and test (physical and chemical) for water and waste water : Part 54 Nickel
3025 (Part 55):2003	Methods of sampling and test (physical and chemical) for water and waste water : Part 55 Aluminium
3025 (Part 56):2003	Methods of sampling and test (physical and chemical) for water and waste water : Part 56 Selenium
3025 (Part 57) : 2005	Methods of sampling and test (physical and chemical) for water and waste water : Part 57 Boron
3025 (Part 59) : 2006	Methods of sampling and test (physical and chemical) for water and waste water : Part 59 Manganese
3025 (Part 60) : 2008	Methods of sampling and test (physical and chemical) for water and waste water : Part 60 Fluoride
3025 (Part 2):2002/ISO 11885:1996	Methods of sampling and test (physical and chemical) for water and waste water : Part 2 Determination of 33 elements by Inductively Coupled Plasma Atomic Emission Spectroscopy
13428:2003	Packaged Natural Mineral Water – Specification (first revision)
14194(Part 1):1994	Radionuclides in environmental samples – Method of estimation : Part 1 Gross beta activity measurement
14194(Part 2):1994	Radionuclides in environmental samples – Method of estimation : Part 2 Gross alpha activity measurement
15302:2002	Determination of aluminium and barium in water by direct nitrous oxide-acetylene flame atomic absorption spectrometry
15303:2002	Determination of antimony, iron and selenium in water by electrothermal atomic absorption spectrometry

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 ($Na_2HPO_4 . 2H_2O$)* – 0.2 M, filter sterilized

B-2.2.4 *Sodium Dihydrogen Phosphate ($NaH_2PO_4 . 2H_2O$)* – 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 ($Mg Cl_2$)* – 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 $Na_2HPO_4 .2H_2O$ 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 0.0005M.

At lower pH pass the water through the membrane. The flow rate shall be 40 litre/hour 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%
Sodium Dodecyl Sulphate (SDS)	1%
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	1M
EDTA	0.5M

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 5000 x g for 30 minutes at 4°C. Add 1/10th volume of 3M sodium acetate and double the volume of cold ethanol to the aqueous layer. Keep the mixture at either at -20°C for over night or in liquid nitrogen for 2-5 minutes. Centrifuge at 10000 x g, for 30 minutes at 4°C. Discard the supernatant and air dry the pellet and dissolve it in 20µl TE buffer.

B-4 COMPLEMENTARY DNA (cDNA) 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 5× reverse transcriptase reaction buffer [250 mM TRIS–HCl (pH 8.5), 40 mM KCl, 150 mM $MgCl_2$, 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 *Micropippette*

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 $MgCl_2$, 0.01% 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

Denaturation for	1.0 min	at 94 °C	35 cycles
Annealing for	1.0 min	at 57 °C	
Extension for	1.3 min	at 72 °C	

Final extension 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 $MgCl_2$, 0.01% 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 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

Denaturation for	1.0 min	at 94 °C	35 cycles
Annealing for	1.0 min	at 42 °C	
Extension for	2.0 min	at 72 °C	

Final extension 72 °C for 7 min.

B-6 AGAROSE GEL ELECTROPHORESIS

B-6.1 Apparatus

B-6.1.1 *Micropippette*

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
0.5M EDTA	50 .00 ml
Distilled water (autoclaved)	300.45 ml

Make the final volume upto 1000 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 1 X

B-6.2.2 *Tracking Dye* (6X Bromophenol blue)
B-6.2.3 *Ethidium Bromide* – 0.5µg/ml

B-6.3 PROCEDURE

Run the PCR amplified product of EV and HAV on 1.5% 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 volts for 30 minutes. Stain the gel with ethidium bromide (0.5 µl/ml) for 20 minutes. 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

Classification of Microscopic Organism	Group and Name of the Organism	Habitat	Effect of the Organisms and Significance
(1)	(2)	(3)	(4)
1 ALGAE	a) *Chlorophyceae* Species of *Coelastrum, Gomphospherium, Micractinium, Mougeotia, oocystis, Euastrum, Scenedesmus, Actinastrum, Gonium, Eudorina Pandorina, Pediastrum, Zygnema, Chlamydomonas, Careteria, Chlorella, Chroococcus, Spirogyra, Tetraedron, Chlorogonium, Stigeoclonium*	Polluted water, impounded sources	Impart colouration
	Species of *Pandorina, Volvox, Gomphospherium, Staurastrum, Hydrodictyon, Nitella*	Polluted waters	Produce taste and odour
	Species of *Rhizoclonium, Cladothrix, Ankistrodesmus, Ulothrix, Micrasterias, Chromulina*	Clean water	Indicate clean condition
	Species of *Chlorella, Tribonema, Clostrium, Spirogyra, Palmella*	Polluted waters, impounded sources	Clog filters and create impounded difficulties
	b) *Cyanophyceae* Species of *Anacystis* and *Cylindrospermum*	Polluted waters	Cause water bloom and impart colour
	Species of *Anabena, Phormidium, Lyngbya, Arthrospira, Oscillatona*	Polluted waters	Impart colour
	Species of *Anabena, Anacystis, Aphanizomenon*	Polluted waters, impounded sources	Produce taste and odour
	Species of *Anacystis, Anabena, Coelospherium, Cleotrichina, Aphanizomenon*	Polluted waters	Toxin producing
	Species of *Anacystis, Rivularia,*	Polluted waters	Clog filters

	Oscillatoria, Anabena		
	Species of *Rivularia*	Calcareous waters and also rocks	Bores rocks and calcareous strata and causes matted growth
	Species of *Agmenellum, Microcoleus, Lemanea*	Clean waters	Indicators of purification
	Species of *Fragillaria, Stephanodiscus, Stauroneis*		Cause discoloration
	Species of *Asterionella, Tabellaria*	Hill streams high altitude, torrential and temperate waters	Taste and odour producing clog filters
	Species of *Synedra* and *Fragillavia*	Polluted waters	Taste and odour producing
	Species of *Nitzchia, Gomphonema*	Moderately Polluted waters	Cause discoloration
	Species of *Cymbela, Synedra, Melosira, Navicula, Cyclotella, Fragillaria, Diatoma, Pleurogsigma*	Rivers and streams impounded sources	Clog filters and cause operational difficulties
	Species of *Pinmularia, Surinella, Cyclotella, Meridion, Cocconeis*	Clean waters	Indicators of purification
	d) *Xanthophyceae* Species of *Botryococcus*	Hill streams, high altitude and temperate waters	Produces coloration
2. ZOOPLANKTON	a) Protozoa *Amoeba, Giardia Lamblia Arcella, Difflugia, Actinophrys*	Polluted waters	Pollution indicators
	Endamoeba, Histolytica	Sewage and activated sludge	Parasitic and pathogenic
	b) Ciliates *Paramecium, Vorticella, Carchesium, Stentor, Colpidium, Coleps, Euplotes, Colopoda, Bodo*	Highly polluted waters, sewage and activated sludge	Bacteria eaters.
	c) Crustacea *Bosmina, Daphnia*	Stagnant polluted waters	Indicators of pollution
	Cyclops	Step wells in tropical	Carrier host of guinea worm

		climate	
3. Rotifers	a) Rotifers *Anurea, Rotaria, Philodina*	Polluted and Algae laden waters	Feed on algae
	b) Flagellates		
	Ceratium, Glenodinium, Peridinium Dinobryon	Rocky strata, iron bearing and acidic waters	Impart colour and fishy taste
	Euglena, Phacus	Polluted waters	Impart colour
	c) Miscellaneous Organisms Sponges, Hydra	Fresh water	Clog filters and affect purification systems
	Tubifex, Eristalls, Chironomids	Highly polluted waters, sewage and activated sludge and bottom deposits	Clog filters and render water unaesthetic
	d) *Plumatella*	Polluted waters	Produces biological slimes and causes filter operational difficulties
	Dreissena, Asellus	Polluted waters	Harbour pathogenic organaisms.