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)

Sl 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, *Max*	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)	*p*H 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, *Max*	1	5	Part 10	—
vi)	Total dissolved solids, mg/l, *Max*	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, *Max*	0.03	0.2	IS 3025 (Part 55)	—
ii)	Ammonia (as total ammonia-N), mg/l, *Max*	0.5	No relaxation	IS 3025 (Part 34)	—
iii)	Anionic detergents (as MBAS) mg/l, *Max*	0.2	1.0	Annex K of IS 13428	—
iv)	Barium (as Ba), mg/l, *Max*	0.7	No relaxation	Annex F of IS 13428* or IS 15302	—
v)	Boron (as B), mg/l, *Max*	0.5	1.0	IS 3025 (Part 57)	—
vi)	Calcium (as Ca), mg/l, *Max*	75	200	IS 3025 (Part 40)	—
vii)	Chloramines (as Cl_2), mg/l, *Max*	4.0	No relaxation	IS 3025 (Part 26)* or APHA 4500-Cl G	—
viii)	Chloride (as Cl), mg/l, *Max*	250	1 000	IS 3025 (Part 32)	—
ix)	Copper (as Cu), mg/l, *Max*	0.05	1.5	IS 3025 (Part 42)	—
x)	Fluoride (as F) mg/l, *Max*	1.0	1.5	IS 3025 (Part 60)	—
xi)	Free residual chlorine, mg/l, *Min*	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, *Max*	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, *Max*	30	100	IS 3025 (Part 46)	—
xiv)	Manganese (as Mn), mg/l, *Max*	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, *Max*	0.5	No relaxation	Clause **6** of IS 3025 (Part 39) Infrared partition method	—
xvi)	Nitrate (as NO_3), mg/l, *Max*	45	No relaxation	IS 3025 (Part 34)	—
xvii)	Phenolic compounds (as C_6H_5OH), mg/l, *Max*	0.001	0.002	IS 3025 (Part 43)	—
xviii)	Selenium (as Se), mg/l, *Max*	0.01	No relaxation	IS 3025 (Part 56) or IS 15303*	—
xix)	Silver (as Ag), mg/l, *Max*	0.1	No relaxation	Annex J of IS 13428	—
xx)	Sulphate (as SO_4) mg/l, *Max*	200	400	IS 3025 (Part 24)	May be extended to 400 provided that Magnesium does not exceed 30
xxi)	Sulphide (as H_2S), mg/l, *Max*	0.05	No relaxation	IS 3025 (Part 29)	—
xxii)	Total alkalinity as calcium carbonate, mg/l, *Max*	200	600	IS 3025 (Part 23)	—
xxiii)	Total hardness (as $CaCO_3$), mg/l, *Max*	200	600	IS 3025 (Part 21)	—
xxiv)	Zinc (as Zn), mg/l, *Max*	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, *Max*	0.003	No relaxation	IS 3025 (Part 41)	—
ii)	Cyanide (as CN), mg/l, *Max*	0.05	No relaxation	IS 3025 (Part 27)	—
iii)	Lead (as Pb), mg/l, *Max*	0.01	No relaxation	IS 3025 (Part 47)	—
iv)	Mercury (as Hg), mg/l, *Max*	0.001	No relaxation	IS 3025 (Part 48)/ Mercury analyser	—
v)	Molybdenum (as Mo), mg/l, *Max*	0.07	No relaxation	IS 3025 (Part 2)	—
vi)	Nickel (as Ni), mg/l, *Max*	0.02	No relaxation	IS 3025 (Part 54)	—
vii)	Pesticides, µg/l, *Max*	See Table 5	No relaxation	See Table 5	—
viii)	Polychlorinated biphenyls, mg/l, *Max*	0.000 5	No relaxation	ASTM 5175*	— or APHA 6630
ix)	Polynuclear aromatic hydro-carbons (as PAH), mg/l, *Max*	0.000 1	No relaxation	APHA 6440	—
x)	Total arsenic (as As), mg/l, *Max*	0.01	0.05	IS 3025 (Part 37)	—
xi)	Total chromium (as Cr), mg/l, *Max*	0.05	No relaxation	IS 3025 (Part 52)	—
xii)	Trihalomethanes:				
	a) Bromoform, mg/l, *Max*	0.1	No relaxation	ASTM D 3973-85* or APHA 6232	—
	b) Dibromochloromethane, mg/l, *Max*	0.1	No relaxation	ASTM D 3973-85* or APHA 6232	—
	c) Bromodichloromethane, mg/l, *Max*	0.06	No relaxation	ASTM D 3973-85* or APHA 6232	—
	d) Chloroform, mg/l, *Max*	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, *Max*	0.1	No relaxation	Part 2	—
	b) Beta emitters Bq/l, *Max*	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	AOAC/ ISO
(1)	(2)	(3)	(4)	(5)
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)	Chlorpyriphos	30	525.2, 8141 A	—
viii)	Delta HCH	0.04	508	—
ix)	2,4- Dichlorophenoxyacetic acid	30	515.1	—
x)	DDT (*o, p* and *p, p* – 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 Water[1)]

(*Clause* 4.1.1)

Sl No.	Organisms	Requirements
(1)	(2)	(3)
i)	*All water intended for drinking*:	
	a) *E. coli* or thermotolerant coliform bacteria[2), 3)]	Shall not be detectable in any 100 ml sample
ii)	*Treated water entering the distribution system*:	
	a) *E. coli* 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)	*Treated water in the distribution system*:	
	a) *E. coli* 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*
1622 : 1981	Methods of sampling and microbiological examination of water (*first revision*)
3025	Methods of sampling and test (physical and chemical) for water and waste water:
(Part 1) : 1987	Sampling (*first revision*)
(Part 2) : 2002	Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy
(Part 4) : 1983	Colour (*first revision*)
(Part 5) : 1983	Odour (*first revision*)
(Part 7) : 1984	Taste threshold (*first revision*)
(Part 8) : 1984	Tasting rate (*first revision*)
(Part 10) : 1984	Turbidity (*first revision*)
(Part 11) : 1983	*p*H value (*first revision*)
(Part 16) : 1984	Filterable residue (total dissolved solids) (*first revision*)
(Part 21) : 1983	Total hardness (*first revision*)
(Part 23) : 1983	Alkalinity (*first revision*)
(Part 24) : 1986	Sulphates (*first revision*)
(Part 26) : 1986	Chlorine residual (*first revision*)
(Part 27) : 1986	Cyanide (*first revision*)
(Part 29) : 1986	Sulphide (*first revision*)
(Part 32) : 1988	Chloride (*first revision*)
(Part 34) : 1988	Nitrogen (*first revision*)
(Part 37) : 1988	Arsenic (*first revision*)
(Part 39) : 1989	Oil and grease
(Part 40) : 1991	Calcium
(Part 41) : 1992	Cadmium (*first revision*)
(Part 42) : 1992	Copper (*first revision*)
(Part 43) : 1992	Phenols (*first revision*)
(Part 46) : 1994	Magnesium
(Part 47) : 1994	Lead
(Part 48) : 1994	Mercury
(Part 49) : 1994	Zinc
(Part 52) : 2003	Chromium
(Part 53) : 2003	Iron
(Part 54) : 2003	Nickel
(Part 55) : 2003	Aluminium
(Part 56) : 2003	Selenium
(Part 57) : 2005	Boron
(Part 59) : 2006	Manganese
(Part 60) : 2008	Fluoride
13428 : 2003	Packaged natural mineral water — Specification (*first revision*)
14194	Radionuclides in environmental samples — Method of estimation:
(Part 1) : 1994	Gross beta activity measurement
(Part 2) : 1994	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 *p*H of the eluent shall be 9.0.

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

B-2.2.9 *McII Vaines Buffer* (*p*H *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 *p*H need not be adjusted. But for the 0.22 micron nitrocellulose membrane adjust the *p*H to 3.5 after adding the aluminium chloride as a coagulant to a final concentration of 0.000 5 M.

At lower *p*H 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 (p*H *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 (*p*H 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 (*p*H 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 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

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 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 (*p*H 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 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

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

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. (1)	*Classification of Microscopic Organism* (2)	*Group and Name of the Organism* (3)	*Habitat* (4)	*Effect of the Organisms and Significance* (5)
i)	Algae	a) Chlorophyceae:		
		1) *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
		2) *Species of* Pandorina, Volvox, Gomphospherium, Staurastrum, Hydrodictyon, Nitella	Polluted waters	Produce taste and odour
		3) *Species of* Rhizoclonium, Cladothrix, Ankistrodesmus, Ulothrix, Micrasterias, Chromulina	Clean water	Indicate clean condition
		4) *Species of* Chlorella, Tribonema, Clostrium, Spirogyra, Palmella	Polluted waters, impounded sources	Clog filters and create impounded difficulties
		b) Cyanophyceae:		
		1) *Species of* Anacystis and Cylindrospermum	Polluted waters	Cause water bloom and impart colour
		2) *Species of* Anabena, Phormidium, Lyngbya, Arthrospira, Oscillatona	Polluted waters	Impart colour
		3) *Species of* Anabena, Anacystis, Aphanizomenon	Polluted waters, impounded sources	Produce taste and odour
		4) *Species of* Anacystis, Anabena, Coelospherium, Cleotrichina, Aphanizomenon	Polluted waters	Toxin producing
		5) *Species of* Anacystis, Rivularia, Oscillatoria, Anabena	Polluted waters	Clog filters

Sl No. (1)	*Classification of Microscopic Organism* (2)	*Group and Name of the Organism* (3)	*Habitat* (4)	*Effect of the Organisms and Significance* (5)
		6) *Species of* Rivularia	Calcareous waters and also rocks	Bores rocks and calcareous strata and causes matted growth
		7) *Species of* Agmenellum, Microcoleus, Lemanea	Clean waters	Indicators of purification
		c) Diatoms (Bacillareophyceae):		
		1) *Species of* Fragillaria, Stephanodiscus, Stauroneis	—	Cause discoloration
		2) *Species of* Asterionella, Tabellaria	Hill streams high altitude, torrential and temperate waters	Taste and odour producing clog filters
		3) *Species of* Synedra and Fragillavia	Polluted waters	Taste and odour producing
		4) *Species of* Nitzchia, Gomphonema	Moderately polluted waters	Cause discoloration
		5) *Species of* Cymbela, Synedra, Melosira, Navicula, Cyclotella, Fragillaria, Diatoma, Pleurogsigma	Rivers and streams impounded sources	Clog filters and cause operational difficulties
		6) *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
ii)	Zooplankton	a) Protozoa:		
		1) Amoeba, Giardia Lamblia Arcella, Difflugia, 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, Peridinium Dinobryon	Rocky strata, iron bearing and acidic waters	Impart colour and fishy taste
		2) Euglena, Phacus	Polluted waters	Impart colour

Sl No.	*Classification of Microscopic Organism*	*Group and Name of the Organism*	*Habitat*	*Effect of the Organisms and Significance*
(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