International Standard ISO 5667/3

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Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples

Qualité de l'eau — Échantillonnage — Partie 3: Guide général pour la conservation et la manipulation des échantillons

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Foreword

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Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples

0 Introduction

This part of ISO 5667 is intended to be used in conjunction with ISO 5667/1 and ISO 5667/2 which deal respectively with the design of sampling programmes and sampling techniques.

1 Scope and field of application

This part of ISO 5667 gives general guidelines on the precautions to be taken to preserve and transport water samples.

These guidelines are particularly appropriate when a sample (spot or composite sample) cannot be analysed on site and has to be transported in order to be analysed in the laboratory.

2 References

ISO 5667/2, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 6228, *Chemical products for industrial use — General method for determination of traces of sulphur compounds, as sulphate, by reduction and titrimetry.*

3 Preservation of samples

3.1 General considerations

Waters, particularly surface waters and above all waste waters, are susceptible to being changed to differing extents as a result of physical, chemical or biological reactions which may take place between the time of sampling and the analysis. The nature and rate of these reactions are often such that, if the necessary precautions are not taken before and during transport as well as during the time in which the samples are preserved in the laboratory before being analysed, the concentrations determined will be different from those existing at the time of sampling.

The causes of variations are numerous; some of these are

— Bacteria, algae and other organisms can consume certain constituents present in the samples; they can also modify the nature of the constituents to produce new constituents. This biological activity affects for example the contents of dissolved oxygen, carbon dioxide, nitrogen compounds, phosphorus and sometimes silicon.

— Certain compounds can be oxidized by the dissolved oxygen contained in the samples or by atmospheric oxygen [for example organic compounds, iron(II), sulfides].

— Certain substances can precipitate out [for example calcium carbonate, metals and metallic compounds such as $Al(OH)_3$, $Mg_3(PO_4)_2$] or be lost to the vapour phase (for example oxygen, cyanides, mercury).

— The pH, conductivity, carbon dioxide content, etc. can be modified by the absorption of carbon dioxide from the air.

— Metals dissolved or in a colloidal state as well as certain organic compounds can be adsorbed or absorbed irreversibly on the surface of containers or solid materials contained in the samples.

— Polymerized products can depolymerize; conversely, simple compounds can polymerize.

The extent of these reactions is a function of the chemical and biological nature of the sample, its temperature, its exposure to light, the nature of the container in which it is placed, the time between sampling and analysis, the conditions (for example rest or agitation during transport) to which it is submitted, etc.

It follows that the variations relative to a particular constituent vary both in degree and rate, not only as a function of the type of water, but also, for the same type, as a function of seasonal conditions.

It must be emphasized moreover that these variations are often sufficiently rapid so as to modify the sample considerably in the space of several hours. It is therefore essential in all cases to take the necessary precautions to minimize these reactions, and in the case of many parameters to analyse the sample with a minimum of delay.

As the variations which take place in the water samples are due to a large extent to biological processes, it is generally necessary to choose from the various possible methods of preservation a method that does not introduce unacceptable contamination.

Even the time for which the preserved sample can be stored before being analysed may change.

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As a guide, it can be said that methods of preservation tend to be less effective in the case of crude sewage than in the case of purified sewage (effluents from biological treatment plants). It has also been observed that the behaviour of various waste water samples during storage is different according to whether the samples have been taken from municipal or industrial sewage-treatment plants.

On the other hand, surface waters and ground waters can in general be stored more effectively. In the case of potable waters, the problem of storage can be solved more easily because these waters are less susceptible to biological and chemical reactions.

Therefore, owing to these variations, which may affect the water samples, it may be necessary, in certain determinations, to take individual samples rather than collective samples and to analyse them immediately at the place of sampling. It should be remembered that the storage of samples for long periods is only possible for the determination of a limited number of parameters.

In spite of numerous investigations which have been carried out in order to recommend methods which will enable water samples to be stored without modification of their composition, it is impossible to give absolute rules in this context which will cover all cases and all situations and which do not have exceptions.

In every case the method of storage shall be compatible with the analytical techniques which will be used. One object of the following is thus to describe the most commonly used techniques.

3.2 Feasible precautions

3.2.1 Filling the container

In the case of samples for the determination of physico-chemical parameters one simple precaution, which is not, however, adequate in all cases, is to **fill the flasks completely and stopper them** in such a way that there is no air above the sample.

This limits interaction with the gas phase and agitation during transport (thus avoiding modifications in carbon dioxide content, and hence variations in pH; hydrogencarbonates are not converted into precipitable carbonates; iron has less tendency to be oxidized, thus limiting colour variations; etc.).

Sample containers, whose contents are frozen as part of their preservation, should not be completely filled.

3.2.2 Use of appropriate containers

The choice and the preparation of a container can be of major importance. ISO 5667/2 gives some guidance on this subject.

However, it should be remembered that the container in which the sample is stored and the stopper should not

— be a cause of contamination (for example borosilicate or soda-lime glass containers may increase the content of silica or sodium)

— absorb or adsorb the constituents to be determined (for example hydrocarbons may be absorbed in a polyethylene container, traces of metals may be adsorbed on the surface of a glass container)

— react with certain constituents in the sample (for example fluorides reacting with glass).

It should be remembered that the use of opaque containers or brown (non-actinic) glass containers can reduce the photosensitive activities to a considerable extent.

Blank samples should be taken, preserved and analysed as a check on the suitability of the choice of container and cleaning procedure.

3.2.3 Cleaning of containers

3.2.3.1 For samples for general chemical analysis

For analysis of trace quantities of chemical constituents of surface or waste water, it is usual to clean new containers thoroughly in order to minimize possible contamination of the sample; the type of cleaners used and the container material vary according to the constituents to be analysed.

For general purposes, new glass containers should be cleaned with water and detergents, to remove dust and packing material. They should then be cleaned with chromic acid-sulfuric acid mixture before being thoroughly rinsed with distilled water.

It may be desired, for environmental or health reasons, to avoid the use of chromic acid. Alternatively, proprietary cleaning agents may be used, provided it has been established that they do not cause sample contamination.

It should be noted that detergents possibly containing phosphates cannot be used if phosphates or surface active agents are to be determined, nor can chromic acid-sulfuric acid mixture be used if trace quantities of sulfate and chromium are to be determined.

Polyethylene containers in general should be cleaned by filling with 1 mol/l nitric or hydrochloric acid, leaving for 1 to 2 days followed by thorough rinsing with distilled or deionized water.

3.2.3.2 For samples for determination of pesticides, herbicides and their residues

In general, brown glass containers should be used because plastics, except polytetrafluorethylene (PTFE), may introduce interferents which can be significant if trace analyses are to be performed.

The containers should be cleaned with water and detergent, followed by thorough rinsing with distilled water, then oven dried and cooled before being rinsed with hexane or petroleum ether. Finally they should be dried with a stream of carefully purified air or nitrogen.

A continuous extraction with acetone for 12 h, followed by a hexane rinse and drying as described above, can also be used.

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3.2.3.3 For samples for microbiological analysis

The containers shall withstand a 160 °C sterilization and shall not produce or release at this temperature any chemicals which would either inhibit biological activity, induce mortality or encourage growth.

When lower sterilization temperatures are used, polycarbonate and heat resistant polypropylene containers may be used. Caps or other stoppers shall withstand the same sterilization temperatures as the containers.

Glass containers should be cleaned with water and detergent, followed by thorough rinsing with distilled water. Then they should be rinsed with nitric acid (HNO_3) followed by thorough rinsing with distilled water in order to remove heavy metals or chromate residues.

A total of 0,1 ml of a 10 % (m/m) solution of sodium thiosulfate ($Na_2S_2O_8$) can be added, for every 125 ml of container capacity, before sterilization. This is to eliminate inhibition of bacteria by chlorine.

3.2.4 Cooling or freezing of the samples

The sample should be kept at a temperature lower than that during filling. Containers should be completely filled.

3.2.4.1 Simple cooling (in melting ice or in a refrigerator between 2 and 5 °C) and storage of the sample in the dark are, in most cases, sufficient to preserve the sample during the transport to the laboratory and for a relatively short period of time before the analysis. Cooling cannot be considered as a means of long term storage, particularly in the case of waste water samples.

3.2.4.2 Freezing (−20 °C) allows in general an increase in the period of storage. Nevertheless, it is necessary to master the freezing and thawing technique fully in order to return the sample to its initial equilibrium after thawing. In this case, the use of plastic containers (for example polyethylene) is strongly recommended.

Glass containers are not suitable for freezing. Samples for microbiological analysis should not be frozen.

3.2.5 Filtration or centrifuging of samples

Suspended matter, sediment, algae and other micro-organisms may be removed, either at the time of taking the sample or immediately afterwards, by filtration of the samples, through filter paper or membrane filter or by centrifuging. Filtration is, of course, not applicable if the filter is likely to retain one or more of the constituents to be analysed. Equally, the filter must not be a cause of contamination and it must be carefully washed before use.

Alternatively, the reason for analysis may involve the separation of soluble and insoluble forms (for example of a metal) by filtration.

Membranes shall be used with caution as various heavy metals and organic material may be adsorbed on the membrane surface, and soluble compounds within the membrane can be leached out into the sample.

3.2.6 Addition of preservatives

WARNING — The use of mercury(II) chloride ($HgCl_2$) shall be avoided, unless absolutely necessary, because of its toxicity to the environment. When it has been used, treatment of the residues in order to recover the mercury shall be provided for (see also ISO 6228, annex C).

It should be remembered that certain preservatives [for example acids, mercury(II) chloride, chloroform] must be used with caution, considering the danger involved in their handling. Operators shall be warned of these dangers and the ways of protecting themselves from them.

Certain constituents can be stabilized by the addition of chemical compounds, either directly to the sample after taking it, or beforehand, to the container when it is still empty.

Various chemical compounds, at concentrations equally varied, have been proposed.

Those most commonly used are

- acids
- basic solutions
- biocides
- particular reagents, necessary for the specific preservation of certain constituents [for example the determination of oxygen, total cyanides and sulfides requires a previous fixation of the sample on site (see the corresponding International Standards on analysis)].

It is essential that the preservatives used do not interfere during the determination; tests intended to check their compatibility are necessary in cases of doubt. Any dilution of the sample with added preservatives should be taken into account during the analysis and the calculation of the results.

It is preferable that the addition of preservatives be made using sufficiently concentrated solutions so that only small volumes are necessary. This enables the corresponding dilution to be disregarded in most cases.

The addition of these agents can also modify the chemical or physical nature of the constituents and it is necessary therefore that these modifications are not incompatible with the objects of later determinations. (For example acidification can solubilize colloidal constituents or solids and shall therefore only be used with caution if the aim of the measurements is the determination of dissolved constituents. If the aim of the analysis is to determine the toxicity to aquatic animals, the solubilization of certain components, particularly heavy metals which are toxic in ionic form, has to be avoided. Samples should therefore be analysed as soon as possible.)

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For some determinations, particularly determinations of trace elements, it is essential to carry out a blank test to take into account possible introduction by the preservatives of an additional amount of the elements to be determined (for example acids can introduce a not insignificant amount of arsenic, lead and mercury). In such a case, the laboratory carrying out the analysis shall retain samples of the preservatives used for the treatment of the water samples for use in the preparation of blank tests.

3.3 Recommendations

As stated in 3.1, it is impossible to give absolute rules for preservation; the duration of preservation, the nature of the container and the efficiency of the preservation processes depend not only on the constituents which have to be analysed and their levels, but also on the nature of the sample. The table **shall therefore be considered only as giving reasonable suggestions.**

In any case in question there shall be no significant difference between the results of a determination carried out immediately and the result obtained after preservation; each analyst should therefore verify, taking into account particularly the method of analysis which he intends to use, whether the suggestions in the table are suitable for the sample with which he is concerned.

Equally, International Standards describing the methods of analysis shall, wherever possible, indicate the recommended methods of preservation.

Moreover, given that possible incompatibility can exist between the analyses to be carried out and the various preservatives and containers possible, it is often necessary to take several samples of the same water and to treat each of them in relation to the analyses for which they are intended. This may result in a compromise between the techniques of preservation which would be most appropriate for each determination taken in isolation. The choice of sample preservation procedure should always be the subject of consultation with the analyst.

4 Identification of samples

Containers holding the samples shall be marked in a clear and durable manner in order to permit identification without ambiguity in the laboratory.

Additionally, it is generally necessary to note, at the moment of sampling, numerous details which will permit a correct interpretation of the information obtained (date and hour of sampling, nature and amount of preservatives added, etc.). Various processes (labels, forms, etc.) allow the practical attainment of these two objectives.

5 Transport of samples

It is obvious that containers holding samples must be protected and sealed in such a way that they do not deteriorate and do not lose any part of their contents during transport. Packaging shall protect the containers from possible external contamination, particularly near the opening, and shall not itself be a source of contamination.

6 Reception of samples in the laboratory

On their arrival in the laboratory, the samples shall, if their immediate analysis is impossible, be preserved under conditions such that any contamination of the outside of the containers is avoided and which prevents any change in their contents.

The use, for this purpose, of refrigerated cabinets or cool and dark places is highly recommended.

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Table — Techniques generally suitable for the preservation of samples

(The information in this table is only a general guide to the preservation of samples. The complex nature of natural and waste waters necessitates, before analysis, a verification of the stability of each type of sample treated according to the methods proposed below.)

A. Physico-chemical and chemical analyses					
1	2	3	4	5	6
Parameter to be studied	**Type of container** P = Polyethylene G = Glass BG = Borosilicate glass	**Preservation technique**	**Place of analysis**	**Maximum recommended preservation time before analysis** (If a preservation period is not specified, it is generally unimportant. The indication "1 month" represents preservation without particular difficulty.)	**Comments**
Acidity and alkalinity	P or G	Cooling to between 2 and 5 °C	Laboratory	24 h	Samples should preferably be analysed at the spot where the sample is taken (particularly for samples high in dissolved gases).
Aluminium filterable[1]	P	Filtration at the place of sampling and acidification of the filtrate to pH < 2	Laboratory	1 month	The filterable[1] aluminium and that adhering to suspended matter may be determined from the same sample.
adhering to suspended matter		Filtration at the place of sampling	Laboratory	1 month	The filterable aluminium on the acidified filtrate and the aluminium adhering to suspended matter may be determined from the filter residue.
total		Acidification to pH < 2	Laboratory	1 month	
Arsenic	P or G	Acidification to pH < 2	Laboratory		
	P	Alkalinization to pH = 12	Laboratory		This technique should be used if arsenides are assumed to be present in samples of domestic or industrial waste water.
Barium	P or BG	See **Aluminium**			Do not use H_2SO_4.
BOD	P or G (Glass is preferable in the case of low BOD.)	Cooling to between 2 and 5 °C and storage in the dark	Laboratory	As soon as possible	
		Freezing to − 20 °C	Laboratory	1 month	
Boron and borates	P		Laboratory	Several months	
Bromides and bromine compounds	P or G	Cooling to between 2 and 5 °C	Laboratory	As soon as possible	Samples should be kept out of direct sunlight.
Cadmium	P or BG	See **Aluminium**			
Calcium	P or G	—	Laboratory	24 h	
		Acidification to pH < 2	Laboratory	Several months	Acidification (do not use H_2SO_4) permits determination of the calcium from the same sample as the other metals.
Carbon dioxide	P or G	—	On site		

1) **filterable:** Denotes that which passes through a filter.

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Table — Techniques generally suitable for the preservation of samples (*continued*)

1	2	3	4	5	6
Carbon, organic	G	Acidification to pH < 2 with H_2SO_4 and cooling to between 2 and 5 °C	Laboratory	24 h	The preservation technique will depend on the method of analysis to be used. The test should be carried out as soon as possible. Freezing (– 20 °C) may be used in certain cases.
Chlorides	P or G	–	Laboratory	Several months	
Chlorine, residual	P or G	–	On site	–	The analysis shall be carried out on site.
Chlorophyll	P or G	Cooling to 4 °C	Laboratory	24 h	
		After filtration and freezing of the residue	Laboratory	1 month	
Chromium(VI)	P or BG	Cooling to between 2 and 5 °C	Laboratory	As soon as possible	
Chromium, total	P or BG	See **Aluminium**			
Cobalt	P or BG	See **Aluminium**			
COD	P or G (Glass is preferable in the case of low COD.)	Cooling to between 2 and 5 °C and storage in the dark	Laboratory	As soon as possible	Acidification is particularly recommended when the COD is due to the presence of organic materials.
		Acidification to pH < 2 with H_2SO_4	Laboratory	2 days	
		Freezing to – 20 °C	Laboratory	1 month	
Colour	P or G	–	On site	–	
		Cooling to between 2 and 5 °C and storage in the dark	Laboratory	24 h	
Conductivity	P or G	Cooling to between 2 and 5 °C	Laboratory	24 h	The test should preferably be carried out on site.
Copper	P or BG	See **Aluminium**			
Cyanides, easily liberatable	P	The preservation technique will depend on the method of analysis to be used.			
Cyanides, total	P	Alkalinization to pH > 12 with NaOH	Laboratory	24 h	
Detergents	See **Surface active agents**				
Diffusion index	See **Turbidity**				
Dry extract	See **Total residue**				
Fluorescein	See **Fluorescent tracers**				
Fluorescent tracers	P (preferably an opaque container)	–	Laboratory	1 month	
Fluorides	P	–	Laboratory	Several months, provided that the sample is neutral	
Greases, oils, hydrocarbons	Glass washed in solvents	Acidification to pH < 2, extraction on site where practicable	Laboratory	24 h	It is recommended that, immediately after sampling, the extraction agent used in the method of analysis be added, or that extraction be carried out on site.
Heavy metals (except mercury)	P or BG	See **Aluminium**			

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Table — Techniques generally suitable for the preservation of samples (*continued*)

1	2	3	4	5	6
Hydrazine	G	Acidification with HCl to 1 mol/l (100 ml per litre of sample) and storage in the dark	Laboratory	24 h	
Hydrogen-carbonates	See **Alkalinity**				
Iodides	Inactinic glass	Cooling to between 2 and 5 °C	Laboratory	24 h	Samples should be kept out of direct sunlight.
		Alkalinization to pH 8	Laboratory	1 month	
Iron(II)	P or BG	Acidification to pH < 2 with HCl and exclusion of atmospheric oxygen	On site	1 week	
Iron, total	P or BG	See **Aluminium**			
Lead	P or BG	See **Aluminium**			Do not use H_2SO_4.
Lithium	P	—	Laboratory	7 days	
		Acidification to pH < 2	Laboratory	Several months	Acidification permits determination of the lithium from the same sample as the other metals.
Magnesium	P or BG	See **Calcium**			
Manganese	P or BG	See **Aluminium**			
Mercury, total	BG	Acidification to pH < 2 with HNO_3 and addition of $K_2Cr_2O_7$ [0,05 % (m/m) final concentration]	Laboratory	Several months	Particular care is needed to ensure that the sample containers are free from contamination.
Nickel	P or BG	See **Aluminium**			
Nitrogen, ammoniacal and Kjeldahl	P or G	Acidification to pH < 2 with H_2SO_4 and cooling to between 2 and 5 °C	Laboratory	24 h	The addition of a bactericide (for example allylthiourea, though the addition of an excess should be avoided) may possibly be considered in order to block the metabolism of the nitrifying bacteria. In this case use a glass container.
		Cooling to between 2 and 5 °C	Laboratory	6 h	For concentrations less than 1 mg/l, it is necessary to carry out analysis on site.
Nitrogen as nitrate	P or G	Acidification to pH < 2 and cooling to between 2 and 5 °C	Laboratory	24 h	For certain waste waters, the sample cannot be preserved and it is necessary to carry out analysis on site.
Nitrogen as nitrite	P or G	Cooling to between 2 and 5 °C	Laboratory	As soon as possible	For certain waste waters, the sample cannot be preserved and it is necessary to carry out analysis on site.
Odour	G	—	Laboratory	6 h	The test should preferably be carried out on site.

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Table — Techniques generally suitable for the preservation of samples (*continued*)

1	2	3	4	5	6
Orthophosphates	B or G	Cooling to between 2 and 5 °C	Laboratory	24 h	The anaylsis should be carried out as soon as possible. The sample should be filtered immediately for the analysis of dissolved phosphate.
Oxygen	P or G	—	On site	—	
		Fixing of the oxygen on site and storage in the dark	Laboratory	4 days at most	Fix the oxygen in accordance with the method of analysis used.
Ozone	—	—	On site	—	
Pesticides, organochloride	G	Cooling to 4 °C	Laboratory	7 days	It is recommended that, immediately after sampling, the extraction agent used in the method of analysis be added, or that extraction be carried out on site.
Pesticides, organophosphorus	G	Cooling to 4 °C	Laboratory	24 h	It is recommended that, immediately after sampling, the extraction agent used in the method of analysis be added, or that extraction be carried out on site.
Petroleum and derivatives	See **Greases, oils and hydrocarbons**				
pH	P or G	—	On site		The test should be carried out as soon as possible and preferably immediately on site after sampling.
		Transportation at a lower temperature than the initial temperature	Laboratory	6 h	
Phenols	BG	Inhibition of biochemical oxidation by $CuSO_4$ and acidification with H_3PO_4 or alkalinization with NaOH to pH > 11	Laboratory	24 h	The preservation technique will depend on the method of analysis to be used.
Phosphorus, total	B or G	—	Laboratory	24 h	
		Acidification to pH < 2 with H_2SO_4	Laboratory	Several months	
Potassium	See **Lithium**				
Putrescibility (methylene blue test)	G	Transportation at a lower temperature than the initial temperature	Laboratory	24 h	The test should be carried out as soon as possible and preferably on site at 20 °C.
Selenium	G or BG	Alkalinization to pH > 11 with NaOH	Laboratory	Several months	
Silicates	P	Filtration and acidification to pH < 2 with H_2SO_4 and cooling to between 2 and 5 °C	Laboratory	24 h	
Silicon, total	P	—	Laboratory	Several months	
Silver	P or BG	See **Aluminium**			Do not use HCl.
Sodium	See **Lithium**				

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Table — Techniques generally suitable for the preservation of samples (*continued*)

1	2	3	4	5	6
Sugars	P or G	Cooling to between 2 and 5 °C	Laboratory	24 h	
		Addition of 30 % (*m/m*) formaldehyde (5 ml per 100 ml of sample) and cooling to between 2 and 5 °C	Laboratory	1 month	This preservation technique is related to the glucose oxidase enzymic determination method.
Sulfates	P or G	Cooling to between 2 and 5 °C	Laboratory	1 week	
Sulfides	P or G	Treatment with 2 ml of 1 mol/l $(CH_3CO_2)_2Zn$ and alkalinization with 2 ml of 1 mol/l NaOH	Laboratory	1 week	
Sulfites	P or G	Fixing on site by addition of 1 ml of a 2,5 % (*m/m*) solution of EDTA per 100 ml of sample	Laboratory	1 week	
Surface active agents, ionic	G	Acidification to pH < 2 with H_2SO_4 and cooling to between 2 and 5 °C	Laboratory	48 h	
Surface active agents, non-ionic	G	Addition of 40 % (*V/V*) formaldehyde to give a 1 % (*V/V*) solution; cool to between 2 and 5 °C and ensure sampling container is completely filled.	Laboratory	1 month	
Suspended and sedimentary matter	P or G	—	Laboratory	24 h	The test should be carried out as soon as possible and preferably on site.
Tin	P or BG	See **Aluminium**			
Total hardness	See **Calcium**			1 to 3 days	
Total residue (dry extract)	P or G	Cooling to between 2 and 5 °C	Laboratory	24 h	The sample should be placed in the capsule used for the test as soon as possible.
Turbidity	P or G	—	Laboratory	As soon as possible	The test should preferably be carried out on site.
Uranium	P or BG	See **Aluminium**			
Zinc	P or BG	See **Aluminium**			

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Table — Techniques generally suitable for the preservation of samples (*continued*)

B. Microbiological analysis					
1	2	3	4	5	6
Enumeration of total bacteria Total coliforms Faecal coliforms Faecal streptococci Salmonella Shigella etc.	Sterile container	Cooling to between 2 and 5 °C	Laboratory	6 h (surface water, sludges and drinking water)	For chlorinated or brominated water the sample shall be collected in a flask containing (before it is sterilized) sodium thiosulfate [in general 0,1 ml of a 10 % (*m/m*) solution of $Na_2S_2O_3$ per 125 ml of sample]. For waters containing heavy metal concentrations greater than 0,01 mg/l, add to the container (before it is sterilized) 0,3 ml of 15 % (*m/m*) EDTA per 500 ml of sample.

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