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Water quality — Sampling for microbiological analysis

Qualité de l'eau — Échantillonnage pour analyse microbiologique

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 19458 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

Appropriate sampling is essential to provide representative samples to the laboratory in charge of testing. Sampling features depend on the objective of sampling, but also on the nature of the sample. Microorganisms are living organisms. In addition, when they are introduced into water, they do not form a perfect solution, but a suspension with an inherent degree of variability.

Sampling objectives may serve different purposes, which are described in the ISO 5667 series of standards (ISO 5667-1, ISO 5667-2 and ISO 5667-3):

a) determination of the compliance of a water with a regulatory quality specification;

b) characterization of any contamination, its level (mean) and its variations:

   1) what is its random variation?

   2) is there a trend?

   3) are there cycles?

c) identification of the sources of pollution.

Regarding the number or frequency of samples, it will vary according to the aim of the sampling.

The minimum number of samples will be low if the mean concentration differs greatly from the specification (much lower or much higher), and the minimum number of samples will be higher if the mean concentration and the specification are close to one another. Similarly, in case b), when looking for a trend: the less obvious the trend, the higher the frequency of sampling (see also Annex A).

Water quality — Sampling for microbiological analysis

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this standard be carried out by suitably trained staff.

1 Scope

This International Standard provides guidance on planning water sampling regimes, on sampling procedures for microbiological analysis and on transport, handling and storage of samples until analysis begins. It focuses on sampling for microbiological investigations.

General information in respect to the sampling from distinct water bodies is given in the respective parts of ISO 5667.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

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

ISO 5667-3, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

3 Sampling point

The sampling site shall provide representative characteristics and account for any vertical, horizontal and temporal variations and shall be identified precisely following the general recommendations of ISO 5667-1 and ISO 5667-2, taking into account additional aspects specific to microbiology.

Sampling points where conditions are unstable should be avoided, and the heterogeneity of the hydraulic system shall be taken into consideration. In studies on the efficacy of disinfection, the sampling point shall be chosen to ensure that the reaction is complete.

EXAMPLE Examples of how the heterogeneity of the system may influence the results are given below.

— It is not equivalent to take a subsurface or a surface sample, or a subsurface sample “contaminated” during recovery through the surface film. In some instances (e.g. lakes, swimming pools), the concentration in the surface film can be 1 000 times higher than in the subsurface.

— All the points of a network are not equivalent, as there may be dead ends and sections where the flow is reduced, particularly if the network is fed from two sources.

— The quality at the outlet of a well-mixed tank is generally the same as in the body of water, but can be quite different from the inlet.

4 Sampling technique

4.1 Personnel

Formal training, training records and determination of competence shall be described for all those who sample, and this information shall be properly documented.

4.2 Sample containers

4.2.1 General

For routine samples (for example, sampling at taps, recreational waters, swimming pool waters), use clean, sterile bottles. The volume of the bottles should be adequate for analysis of all requested parameters.

For sampling by immersion in clean waters, use bottles that are sterile both inside and out and protected, for example, by kraft paper (to keep dry after autoclaving), aluminium foil or by plastic outer bags.

If not autoclavable, sterilization with gamma rays or by ethylene oxide may be used. The bag can then be opened just before sampling and can also serve as a glove to hold the bottle to provide maximum asepsis before being placed on a pole or other sterilizable sampling apparatus.

Alternatively, the outside of sample bottles may be disinfected immediately prior to immersion by a suitable disinfectant such as isopropanol (4.3.1.1) and allowed to dry before use. This is not suitable for analysis of spore-forming bacteria.

In most cases, 500 ml bottles are sufficient, as less than five categories of microorganisms are measured, each involving inoculation of a maximum of 100 ml.

In some cases, larger volumes are necessary, e.g.:

— for bottled water analysis (250 ml per parameter);

— for *Legionella* spp. or *Salmonella* spp. (up to 1 l);

— for viruses, *Giardia* cysts, *Cryptosporidium* oocysts, amoebae in clean waters, from 10 to several hundred litres or more are examined. Usually, a concentration step is made on site using a cartridge filter which is then transported to the laboratory.

Bottles can be made of glass or various plastics (polypropylene, polystyrene, polyethylene, polycarbonate). Usually glass is preferred for re-use, and polyethylene is used as disposable.

Adhesion to surfaces can lower the detection of microorganisms, and the critical tangential surface tension γ has to be considered if a non-standard material is used [13].

Closures can be a ground glass or plastic stopper for glass bottles, a plastic press-on lid for plastic bottles or jars, or a plastic or metal screw cap for either. Bottle openings closed with plastic or glass stoppers should be further protected from contamination by, e.g. aluminium foil.

When larger volumes are necessary for the assay of, for example, viruses, *Salmonella* spp., amoebae, *Cryptosporidium* oocysts, *Giardia* cysts, it is sometimes necessary to analyse tens of litres or hundreds of

litres. To avoid the difficulties of handling, refrigerating and agitating such volumes, a concentration step *in situ* (by flocculation, centrifugation or filtration) is recommended. Peristaltic pumps can be used with sterile tubing.

NOTE 1 Metal caps, especially aluminium, can produce toxicity when autoclaved. This can be prevented by incorporating a heat-resistant leak-proof liner.

NOTE 2 Certain materials can also give toxic by-products when heat sterilized, even in a dry oven, or induce pH changes.

NOTE 3 Some brands of cotton wool used to make plugs for glassware may become toxic if they are heated for too long at too high temperatures.

NOTE 4 Press-on plastic lids attached to the bottle or jar have several advantages in that they are as leak-proof as screw-caps, and the lids can stand open, which facilitates filling and pipetting. When open, the lid remains linked to the bottle, so bottles and closures are kept together, and the lid is also protected from contamination.

4.2.2 Sterilization of bottles

If re-used, clean glass bottles and their closures with a non-toxic, phosphorus-free detergent, followed by a thorough rinse with deionized or distilled water.

Autoclave bottles at (121 ± 3) °C for at least 15 min. Keep the closure of the bottles loose, to allow the steam to replace all the air during the temperature rise, and to prevent plastic bottles from collapsing when cooling. Tighten screw caps after sterilization. Autoclave glass stoppers separately from the bottle, or use a paper or aluminium separator to prevent the stopper sticking on cooling.

If necessary, sterilize bottles in a dry oven for at least 1 h at (170 ± 10) °C. Separate ground glass stoppers from the neck by a paper strip or a piece of string to avoid jamming during cooling. The bottles should be traceable to the sterilization date.

Control the effectiveness of the sterilization process by chemical or biological indicators.

When sterilization is not possible with any other means, disinfect by immersing open bottles in boiling water for at least 30 min. Immediately after boiling, empty the bottles and close them with boiled caps and wrapped in clean paper.

NOTE 1 Polyethylene bottles can be sterilized by exposure to ethylene oxide gas, but, because of its toxicity, the procedure is carried out in specialized facilities and time allowed for desorption of the ethylene oxide. It is therefore not used as a routine laboratory procedure.

NOTE 2 Exposure to gamma rays produced by a ^{60}Co or ^{137}Cs source or to accelerated electrons of sufficient energy (1×10^4 Gy to 2×10^4 Gy) is a very efficient sterilization technique, available in specialized installations. There is no residual antibacterial activity, but some materials may be altered by polymerization after repeated irradiation.

4.2.3 Inactivation of disinfectants

To assess the microbiological quality of water disinfected by an oxidant (e.g. chlorine, chloramine, bromine or ozone), stop the action of the oxidant as soon as the sample is taken. Add a reducing agent such as sodium thiosulfate to the sample bottles.

The theoretical mass of sodium thiosulfate (pentahydrate) necessary to inactivate 1 mg of chlorine is 7,1 mg. Thus, 0,1 ml of sodium thiosulfate pentahydrate solution (4.3.1.2) is added for each 100 ml of bottle capacity. This will inactivate at least 2 mg/l and up to 5 mg/l of free chlorine residual, depending on inactivation dynamics, which is sufficient for the majority of samples.

In certain circumstances, such as foot baths in swimming pools, disinfection measures (e.g. *Legionella* eradication in drinking water distribution systems), higher chlorine concentrations can be found and a proportionately higher dosage of sodium thiosulfate will be necessary.

Sodium thiosulfate is not destroyed by autoclaving or dry heat. Ensure that the pH of the sodium thiosulfate solution is around neutral (low pH can cause decomposition).

Sodium thiosulfate has no effect on the sample and can be used for non-chlorinated waters too.

NOTE It has been claimed that *Legionella* are sensitive to sodium and that potassium thiosulfate is preferable, but no adverse effect of sodium has been detected at the concentration used to inactivate usual chlorine concentrations.

For other disinfectants, corresponding inactivation measures need to be taken. If inactivation is not possible or feasible, it has to be reported.

Chelating agents have been recommended to protect bacteria from the toxic action of heavy metals such as copper or zinc. Ethylene dinitrilotetraacetic acid (EDTA) or sodium nitrilotriacetate (NTA) ($Na_3C_6H_6NO_6$) can be used as a filter-sterilized solution at a final concentration of about 50 mg per litre but should only be added when necessary (e.g. water treated with silver or copper). Silver can also be inactivated by sodium sulfide. Add 1 ml of a sodium sulfide solution (4.3.1.3) to 1 l of sample.

4.2.4 Quality control of sample bottles

4.2.4.1 Testing of sterility

The laboratory shall ensure the sterility of the sample bottles, whether they are prepared in-house or commercially, whether they are made of glass or of plastic. Commercially prepared bottles should be delivered with a certificate of sterilization as a condition for acceptance, and sterility tests are also advisable on the batch in use. This relates to the batch of bottles after labelling, addition of inactivation agents where relevant, and storage.

The sterility of bottles can usually be guaranteed by control of the sterilization process. If not, the sterility of the containers should be tested.

EXAMPLE The following are examples of testing procedures (usually performed a rate of 1 per 100 bottles):

a) “Roll bottle” method

This consists of introducing 20 ml or 50 ml of melted nutrient agar (plate count agar) into the test bottle and lining the walls with agar by rotating the bottle while cooling (under a trickle of water if necessary). Incubation at (22 ± 2) °C for five days should give no visible growth.

b) Liquid broth method

This consists of placing 20 ml to 50 ml of thioglycollate or other nutrient broth inside the bottle, rolling the bottle to wet the walls and incubating at (22 ± 2) °C for five days. No turbidity should appear if sterile.

4.2.4.2 Testing for the presence of inactivating agents

The presence of thiosulfate may be checked by an iodometric method:

$$I_2 + 2\ S_2O_3^{2-} \longrightarrow 2\ I^- + S_4O_6^{2-}$$

Add 10 ml distilled water to the bottle and titrate with iodine solution (4.3.1.4), using starch or thiophene as an end point titration agent.

4.2.4.3 Testing for residual toxicity in sample bottles

Residual toxicity in sample containers may result from the washing procedure of glassware, from the release of components or additives from plastic bottles and also from the sterilization process. Routine use of glass or polyethylene bottles does not require a regular check for toxicity, but if in any doubt, test according to Geldreich, 1975 [8] (for example).

4.3 Reagents, apparatus and materials

4.3.1 Reagents

4.3.1.1 Ethanol, volume fraction $\varphi(C_2H_5OH) = 70\ \%$, **isopropanol**, volume fraction $\varphi[(CH_3)_2\ CHOH] = 70\ \%$, or **hypochlorite solution**, $\rho(CLO^-) \approx 1$ g/l.

4.3.1.2 Sodium thiosulfate pentahydrate solution, $\rho(Na_2S_2O_3 \cdot 5H_2O) = 18$ mg/ml.

4.3.1.3 Sodium sulfide solution, $\rho(Na_2S) = 0{,}1$ mg/ml.

4.3.1.4 Iodine solution, $c(I_2) = 0{,}05$ mol/l.

4.3.2 Apparatus and materials

In addition to sample containers, the following items may be necessary.

4.3.2.1 Soap and towels.

4.3.2.2 Gas blow lamp and **refill**.

4.3.2.3 Jars or beakers, disinfecting wipes.

4.3.2.4 Lighter, matches.

4.3.2.5 Markers, pencils, labels.

4.3.2.6 Spanners, pliers, screwdrivers, knife.

4.3.2.7 Icebox and ice or ice packs, portable refrigerators or refrigerated compartments in vehicles.

4.3.2.8 Thermometer or temperature recorder.

4.3.2.9 Ballasted bottle-carrier or equivalent, with rope or chain (preferably stainless steel, at least the bottom part).

4.3.2.10 Pole or long forceps or samplers adapted to various depths.

4.3.2.11 Maps, list of sampling points, sampling forms.

4.3.2.12 Vehicle and papers, identity or authorization card.

4.3.2.13 Waterproof (safety) boots.

4.3.2.14 Apparatus to measure pH, chlorine, dissolved oxygen, conductivity.

4.3.2.15 Sterile gloves.