



ENVIRONMENT AGENCY

The Microbiology of Drinking Water (2010) - Part 2 – Practices and procedures for sampling

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet updates and replaces the earlier version published in 2002 and contains details of the practices and procedures that should be adopted for taking samples for microbiological analysis.

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Whilst specific commercial products may be referred to in this document, this does not constitute an endorsement of these products but serves only as illustrative examples of the type of products available. Equivalent products may be available and it should be understood that the performance of methods might differ when other materials are used and all should be confirmed by validation of the methods.

Within this series there are separate booklets, each dealing with different topics concerning the microbiology of drinking water. Booklets include

The Microbiology of Drinking Water (2002)

Part 1 - Water quality and public health

Part 3 - Practices and procedures for laboratories (currently undergoing revision)

Part 10 - Methods for the isolation and enumeration of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment.

The Microbiology of Drinking Water (2004)

Part 11 - Taste, odour and related aesthetic problems

Part 12 - Methods for micro-organisms associated with taste, odour and related aesthetic problems.

The Microbiology of Drinking Water (2006)

Part 9 - The isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube-most probable number techniques

The Microbiology of Drinking Water (2007)

Part 7 - Methods for the enumeration of heterotrophic bacteria (currently undergoing revision)

Part 13 - The isolation and enumeration of aerobic spore-forming bacteria by membrane filtration

The Microbiology of Drinking Water (2009)

Part 4 - Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7)

Part 14 - Methods for the isolation, identification and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts

The Microbiology of Drinking Water (2010)

Part 2 - Practices and procedures for sampling

Part 5 - The isolation and enumeration of enterococci by membrane filtration

Part 6 - Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration

Part 8 - Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa* by membrane filtration

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments and biota.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

In the procedures described in each method any reference to the tolerances to be adopted with respect to, for example the amount or volume of reagents to be used is left to the discretion of the laboratory. These tolerances should be as low as possible in order to satisfy stringent performance criteria. Tolerances of between 1 - 5 % have been shown to be satisfactory for most purposes. Lower tolerances should result in improved precision.

In the methods described, for example for wavelengths, storage conditions, concentrations of the same or similar reagents, etc, differences may be noted. This information is provided by individual laboratories operating under their own management systems and is dependent on specific conditions pertaining to each laboratory. It is assumed this information is supported by sufficient data to justify its inclusion. Users intending to use or vary the quoted wavelengths, storage conditions, concentrations, etc, should ensure they are appropriate to their own laboratory and verify their application to demonstrate

appropriate performance of the method. In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency.

Methods are produced by panels of experts in the appropriate field, often in co-operation with working groups and the main committee. The names of those members principally associated with these methods are listed at the back of this booklet. A report describing all SCA activities for the period 1 July to 30 June is produced annually, and is available from the Agency's web-page (www.environment-agency.gov.uk/nls).

Users should ensure they are aware of the most recent version of the draft they seek. If users wish to receive copies or advance notice of forthcoming publications, or obtain details of the index of methods then contact the Secretary on the Agency's internet web-page or by post, see address listed at the back of this booklet.

Great efforts are made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood

Secretary

February 2010

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

Practices and procedures for sampling

1 Introduction

1.1 Design of sampling programmes

Whenever a sample of water is taken for analysis it should be recognised that irrespective of the volume of water sampled, i.e. submitted to the laboratory for analysis, this volume represents only a very small fraction of the water being sampled, whether being stored, produced or distributed. Sampling programs should be designed to take into account many factors, including but not being restricted to;

- i) the reason for taking the sample;
- ii) the location of the site, and whether it is appropriate, i.e. fit for purpose;
- iii) the frequency of sampling;
- iv) the sampling technique used to obtain a representative sample;
- v) the correct use of suitable containers for collecting the samples;
- vi) the storage conditions of samples once taken and their subsequent transportation to the laboratory; and
- vii) the analytical methodology and performance requirements.

Detailed advice on the design of sampling programs can be found elsewhere⁽¹⁻³⁾. Guidance on regulatory sampling programs for drinking water can be obtained elsewhere for England and Wales⁽⁴⁻⁸⁾, Scotland⁽⁹⁾ and Northern Ireland⁽¹⁰⁾.

Whilst undertaking sampling for microbiological parameters, the health and safety of sampling staff should be of paramount importance, as well as maintaining the integrity of the sample. The body of water being sampled should also be safeguarded. For most microbiological examinations, a single discrete sample is often all that is required. However, for certain microbiological parameters, for example *Cryptosporidium*, continuous sampling techniques are usually required.

1.2 Health and safety

Before taking a sample for microbiological examination, the need for undertaking a risk assessment should be considered in order to ensure the safety of sampling staff associated with the sampling process. Considerations include but are not restricted to

- i) whether staff should work alone,
- ii) the dangers of working at height or in confined spaces,
- iii) the entry into potentially noisy environments,
- iv) the proximity of an open, potentially hazardous body of water, and
- v) the risks of handling chemicals and equipment required for the sampling process.

Each sampling occasion should be assessed and it may be necessary to undertake a site survey prior to commencement of sampling operations.

1.3 Samples

The taking of a sample in the correct manner, in a suitable container, and transporting and storing it appropriately before beginning the analysis, is a vital part of any microbiological examination. If the sample is, for example

- i) not representative of the material under investigation,
- ii) contaminated during sampling,
- iii) incorrectly stored before and during transport to the laboratory and subsequently at the laboratory, prior to analysis,
- iv) contaminated during analysis

then, irrespective of how sophisticated or reliable the analytical method or how well the analysis is carried out, the reported analytical result will probably be misleading and open to mis-interpretation.

The sampling procedures described in this booklet are equally applicable to public and private water supplies. Before sampling any water, it may be important for staff to be aware of relevant details of the source of the water, treatment and/or distribution arrangements that may be pertinent, and any other factors, in order to ensure that the water sampled and submitted to the laboratory is representative of the bulk of water from where the sample is taken. In some cases, specific sampling arrangements may be necessary.

2 The sampling manual

Organisations undertaking water sampling should set out in a sampling manual specific guidance on sampling procedures, sample handling techniques, preservation and transportation details and information on on-site analytical methods. This manual should be available to all sampling staff who should be aware of its contents. As a minimum, the sampling manual should include:

- (i) A description of all sample bottles or containers to be used for specific parameters to be determined. In addition, the conditions under which bottles should be prepared and stored should be described. Any preservatives required for stabilising the sample should be detailed as should the expiry date or shelf life of the preservatives. Also, the period of time for which the bottles or containers may be stored should be given.
- (ii) A full description of the sampling procedures to be used at each location including, where necessary, the order in which the samples are to be taken.
- (iii) Advice on how to avoid contaminating the sample during sampling.
- (iv) Advice on storing and transporting samples to the laboratory, and if necessary storing samples in the laboratory prior to analysis.

3 Training of sampling staff

All sampling staff should receive appropriate training, which should be subject to verification, before being allowed to work unsupervised. Once trained, the performance of sampling staff should be subject to review. A training record for each person undertaking sampling should be documented, and contain details such as, for example;

- (i) the training received;
- (ii) the verification process;
- (iii) reviews, assessments or audits (together with dates) undertaken to ascertain the competence of sampling staff and any relevant details of conclusions reached or recommendations made;
- (vi) any other relevant information.

Before undertaking any sampling, sampling staff should receive specific hygiene awareness training. Sampling staff should also possess an understanding of the basic principles and practices of water production, distribution and quality. In addition, sampling staff should demonstrate a basic knowledge of relevant chemical and microbiological analytical procedures. All sampling staff should be trained and assessed in the practices and procedures they use⁽¹¹⁾.

Independent audits should be undertaken to verify that suitable sampling operations are used and this process should include procedures for sample collection, storage and transportation, as well as checks carried out for analytical quality control and on reagents and equipment used for on-site testing, such as pH, turbidity, conductivity and free and combined residual chlorine determinations.

4 Sample containers

For most routine microbiological testing of indicator bacteria, sample containers generally consist of bottles of a capacity of between 300 - 500 ml. Larger volume containers, such as 10 litre carbuoys may be necessary, for example for the examination of specific protozoan parasites, viruses or for special investigations.

Containers for microbiological sampling should be used exclusively for this purpose and should be made of suitable pre-sterilised disposable plastic, or of good quality soda or borosilicate glass. All such containers should be free from toxic substances. If disposable containers are not used, for example glass bottles, containers should be clean and sterilised before use. Glass containers should not be used on food manufacturing premises or at other locations, such as swimming pools, where their use may present a potential hazard, for example if they break. In addition, containers showing any signs of defect, or which appear visibly sub-standard, should not be used, for example those which are damaged or dirty, etc.

Sample containers should always be labelled (see section 4.2) in a manner that enables staff to clearly identify

- i) the sample,
- ii) the precise location from where the sample was taken,
- iii) the time and date the sample was taken, and
- iv) any other relevant information regarding the sample, including, if necessary, details of the required analysis.

Such information may be written directly on the label or be readily available where electronic data recording devices have been used.

Prepared containers should be stored in a suitable location that is dry, secure, organised and well maintained in order to prevent accidental contamination prior to use.

4.1 Container preparation

4.1.1 Re-useable containers

When the microbiological analysis has been completed, any remaining sample should be discarded appropriately, and containers and caps that are to be re-used, should be washed thoroughly with phosphate-free, non-toxic detergent, followed by thorough rinsing with distilled or deionised water, or water of similar quality. After rinsing, containers should be allowed to drain. Laboratory-based washing machines undertake these procedures automatically and provide suitable washing cycles to accommodate many containers.

Treated waters often contain traces of disinfectants such as chlorine or chloramines. Hence, prior to taking a sample and before commencement of analysis, sufficient sterile sodium thiosulphate solution should be added to all empty containers used for microbiological purposes. This addition should neutralise any residual disinfectant that may be present in the sample. This is to ensure that disinfection processes do not continue to occur within the sample once collected in the container. The addition of sodium thiosulphate solution is not required when *Cryptosporidium* determinations are to be undertaken⁽¹²⁾.

At pH values normally occurring in water supplies, i.e. pH values between 6.5 and 9.5, the addition of sodium thiosulphate pentahydrate solution (at a concentration of 18 mg/l) should neutralise up to 5 mg/l of free and combined residual chlorine contained in the sample. It is reported⁽¹³⁾ that *Escherichia coli* (*E. coli*) and coliform bacteria are not adversely affected by sodium thiosulphate at this concentration.

To the empty, clean and dry container, add approximately 0.1 ml of a freshly prepared 1.8 % m/v sodium thiosulphate pentahydrate solution for each 100 ml of sample to be collected. Containers should then be capped or stoppered. Where necessary (for example, those bottles with glass-stoppers) the stopper should be covered with suitable additional material (for example, aluminium foil). Once capped or stoppered, the container should be sterilised either by autoclaving at 121 °C for 20 minutes, or by heating to 160 °C in a fan-assisted oven and maintaining this temperature for 60 minutes. The sterilised containers should be removed from the autoclave or oven, allowed to cool and stored appropriately.

Periodically, after sterilisation, a check should be carried out to ensure the efficacy of the sodium thiosulphate in neutralising an aqueous solution of chlorine. For example, to a 500 ml sterile sample bottle containing 0.5 ml of 1.8 % m/v sodium thiosulphate solution add 500 ml of chlorine solution, at a concentration of 5 mg/l, and determine the free and combined residual chlorine content. The number of checks and the frequency with which they are carried out should be appropriate to the number of containers sterilised and stored. If checks reveal that the sodium thiosulphate solution is not sufficient to neutralise the disinfectant, the batch of containers should be discarded and the cleaning and sterilising process repeated.

Sample containers may possess plastic caps, or metal caps with silicone rubber liners, that can be autoclaved. If bottles with ground glass stoppers are used, a thin strip of material, for example grease-proof paper or aluminium foil should be inserted between the stopper and the

neck of the bottle, before commencing sterilisation. This insertion prevents the stopper fusing with the neck of the bottle, and enables the stopper to be easily removed following sterilisation, and may prevent subsequent damage to the bottle when the bottle cools.

For each batch of bottles, the sterilisation cycle should be monitored/verified, for example using calibrated temperature probes placed at appropriate positions within the batch being sterilised. This should demonstrate whether all bottles within a single batch of bottles have each received appropriate heat treatment. Whilst the use of indicator tape alone may not be sufficient to demonstrate this process, it is a useful visual aid of heat treatment. (This process provides evidence to show that the stacking of bottles, sterilisation process, etc shows the batch of bottles to be satisfactory). Indicator tape is usually referred to as autoclave tape and is primarily used for wet heat sterilisation although it can be used for dry heat sterilisation.

Bottles should only be released for sampling when the sterilisation process has been shown to be satisfactory for the batch. Sterilising equipment should be serviced at regular intervals and calibration undertaken, to ensure for example correct cycle-time, pressure attained and temperature performance etc, accord with manufacturer's instructions.

Each batch of (correctly sterilised) containers should be clearly identifiable, for example with a batch code, and including an expiry date, i.e. the date by which the sample container should be used. Sample containers that are to be re-used should normally be used within three months of the addition of sodium thiosulphate solution and sterilisation. Any containers that are not used before the expiry date should be removed from storage. Containers can then be re-cleaned and sterilised, or discarded, as appropriate.

4.1.2 Disposable containers

Disposable microbiological containers, i.e. containers that are used only once, may be purchased as sterile containers (usually by irradiation) containing the correct amount of disinfectant neutralising reagent. These containers usually comprise a plastic bottle with cap, together with some means of indicating whether the cap has been tampered with. Where a disposable, sterilised container is to be used, the cap should be intact and the bottle sealed, and the cap unscrewed only at the time of sampling. If, just prior to sampling, the cap is broken or appears to have been tampered with, the container should be discarded. A statement or certificate should accompany the container to show its suitability in terms of having undergone a satisfactory sterilisation process and that it contains the correct amount of disinfectant neutralising reagent. Periodically, a visual check on these containers should be carried out to ensure they are not damaged or defective, and that they contain neutralising agent.

It may be necessary or advisable, that an appropriate number of these containers should be checked to confirm that they are sterile and to ensure that they contain the correct amount of neutralising reagent. (See neutralising check for re-usable containers, section 4.1.1).

The expiry date of disposable sterilised containers containing disinfectant neutralising reagent is often specified by the manufacturer and is typically one year, when stored appropriately.

4.1.3 Container storage and quality control

Sterilised containers should be stored in a dedicated area that is clean, dry and not exposed to extremes of temperature.

A documented quality control system should be in place and details of checks regarding sterilisation, neutralising reagent, storage and expiry date should be recorded. In addition, if microbiological containers are despatched to remote locations where these containers are stored and used, similar checks should also be carried out and details recorded. This documented quality control system should also contain details of actions to be taken if checks reveal an adverse number of results are reported.

Irrespective of the type of microbiological container used, staff should periodically check⁽¹⁴⁾ bacteriological samples submitted to the laboratory in order to ensure the absence of detectable levels of chlorine or other disinfectant in the sample. This check should be carried out, as soon as possible, on any remaining sample left in the container after the volume of sample has been removed for the microbiological analysis. If a detectable level of disinfectant is found, further checks should be carried out on the same batch of bottles and if available from the same sampler. If a sample is found to contain chlorine or other disinfectants, the results should not be reported. A further sample should be obtained and submitted for testing.

4.2 Container labels and sampling records

All microbiological containers should be suitably labelled. Self-adhesive labels have been found suitable. For containers that are to be re-used, the labels should be removed after the analysis has been completed and results reported, prior to subsequent washing and sterilisation. Labels may be pre-printed and may include bar-coding, but the information on them should be in a permanent form, for example using water-resistant ink. Information that is hand-written on labels should also be permanently recorded. A documented system should be in place to ensure that appropriate sampling information is available for all samples, and that suitable records are kept. This information should include for example:

- (i) a unique laboratory sample reference number or code;
- (ii) the date and time of sampling;
- (iii) the exact location of the sampling point;
- (iv) the type of water being sampled (for example, raw, filtered, treated, distribution etc);
- (v) the reason for taking the sample (for example, compliance, operational, complaint etc);
- (vi) the identity of the person taking the sample;
- (vii) the free and combined residual chlorine concentration, if appropriate;
- (viii) details of the analysis required;
- (ix) any other relevant details;

This information may be recorded on a sample worksheet or in a log-book.

The sample label may contain some or all of this information, and where appropriate, should include a cross reference to the sampler's worksheet, record or log-book, including any electronic recording device, where all relevant information is recorded and stored at the time

the sample is taken. Where a number of samples are collected from the same location or sample point, each container should be uniquely labelled.

Any unusual event or situation that is observed or encountered during the sampling operation should be recorded at the time of sampling. This includes any information relevant to taking the sample or sampling conditions occurring at the time. This kind of information is often of great value to making a correct interpretation of microbiological results. This information may include factors such as :

- the type and condition of the sampling tap used;
- the presence of anti-splash attachments or inserts;
- the method of disinfection, if appropriate;
- the aesthetic quality of the water sampled;
- weather conditions (especially if sampling outdoors);
- any unusual features observed or encountered during sampling; and
- the results of on-site tests performed at the time of sampling.

The recording of this information may facilitate an assessment of the information collated over a sustained period of time, on how such factors influence the sampling process .

The location of the sampling point should always be identified in sufficient detail to enable a repeat sample to be taken from the same place. Fixed sample points, see Figure 1, such as those at treatment works and service reservoirs, should have a permanent notice or label attached to them, or in proximity to them, indicating for example, the exact location of the tap and possible reasons for its use and the nature or type of the water being sampled. Other details that may be useful to sampling staff might include information on the time required to adequately flush water to waste before sampling.

Figure 1 Example of a labelled fixed sampling point



5 Sampling procedure

Physical and chemical testing may need to be carried out on-site at the same time that microbiological sampling is undertaken. Since different types of sample and sampling bottles are used for different parameters, the correct order of sampling and associated on-site testing is essential in order to ensure that appropriate samples are taken. In addition, it is important that steps are taken to eliminate or reduce the possibility of cross-contamination occurring between the different sampling operations. Whilst samples for physical and chemical determinations do not require the use of “aseptic techniques”, their application is essential when taking microbiological samples.

5.1 Order of sampling

The order of sampling is:

- (i) Samples that need to be taken first are those that are often referred to as “first draw samples”. In these cases, the tap should not have been used that day prior to sampling the water, and should contain water that has been left standing for long periods of time for example over-night in associated pipe work. These types of samples include those taken at customers’ premises for the analysis of, for example lead, copper and nickel. As soon as the tap is turned on, a suitable flow rate should be used to collect sufficient water in a suitable container which is then sealed for submission to the laboratory for analysis.
- (ii) After taking the “first draw sample”, the tap should be left on and water allowed to run to waste in order to remove any debris, sediment and/or biofilm contained within the tap and associated pipe work. Depending on individual circumstances, this flushing process may take several minutes or longer.
- (iii) Samples used for appropriate on-site physical and chemical tests should then be taken. When the tap and associated pipe work are free of debris, sediment and/or biofilm sufficient water is collected in a suitable container. This sample may be used for the analysis of, for example, residual chlorine, pH, temperature, turbidity and electrical conductivity, and results recorded on-site.
- (iv) Samples used for physical and chemical testing (including tests for organic substances) and samples used for invertebrate testing, that are to be carried out at the laboratory, should be taken next. These samples can be taken before or after the on-site tests have been completed but should be taken after water has been allowed to run to waste clearing the tap and associated pipe work of debris, sediment and/or biofilm.
- (v) When stages (i) to (iv) have been completed, including on-site tests, the tap should be turned off and then disinfected. The procedure used for disinfecting the tap should take into account local practices and safety considerations. The application of 1 % available chlorine (equivalent to 10000 mg/l as chlorine) sodium hypochlorite solution to the tap or flaming the tap (using a small proprietary propane or butane burner) are two commonly used procedures. There are advantages and disadvantages using both

procedures (see sections 5.2.1 and 5.2.2) and local policy should dictate which procedure should be used.

- (vi) Following the disinfection process referred to in stage (v), the tap should be turned on and water allowed to run to waste in order to remove any disinfectant or heated water contained within the tap or associated pipe work. Depending on individual circumstances, this flushing process may take several minutes or longer. Where sodium hypochlorite solution has been used, the free residual chlorine concentration should be determined again, to ensure that concentration levels have returned to those previously determined. Bacteriological samples and, if required cotton wool swabs, should then be taken.

Microbiological samples should always be collected in sterile containers containing sufficient disinfectant neutralising reagent. The application of aseptic techniques (i.e. minimising contamination) is required in order to take satisfactory microbiological samples. Prior to taking the sample, free and combined residual chlorine concentrations should be at those levels previously determined. The flow of water leaving the tap should be adjusted to a steady and gentle flow rate such that it does not cause splashing as the container is filled, and does not change while the sample is being collected. If taps are used that can be swivelled, then these should not be moved once the flow rate has been set. If a change in the flow rate is experienced, the container should be discarded and a new one used for sampling, and water from the tap should be allowed to run to waste, as in (ii) above, to ensure that any debris, sediment and/or biofilm that may have been dislodged in the tap and associated pipe work, as a result in the change of flow of water, is removed before sampling recommences.

To fill the container, it should be held in one hand and the stopper or cap removed with the other hand, whilst at the same time taking care not to touch the top or neck of the container. The container should not be rinsed out, and the stopper or cap should not be placed on any surface. From a steady and gentle stream of water from the tap, the container should be filled, leaving a small air gap or head space. Splashing should be avoided and the container should not be allowed to overflow. After the sample has been collected, the container should then be sealed immediately with the stopper or cap, again taking care not to touch the top or neck of the container. The small air gap or head space should be sufficient to enable the sealed container to be inverted and the contents mixed thoroughly following collection of the sample. The container and its contents should then be kept cool, for example by placing it in an insulated cool box or refrigerator, and transported to the laboratory (see section 7). If, whilst taking the sample, contamination occurs, or is suspected, the sample and container should be discarded and another container used to take a new sample. Similarly, if any damage to the sample container or lid is noticed, another container should be used and the damaged one discarded.

If swab samples are required, depending on the circumstances, for example for investigation purposes, then it may be necessary to take the samples in a different order. In some cases swab samples may be required in stage (i) and again after stage (ii). Section 5.4 gives details on how to take swab samples.

5.2 Procedures for disinfection

Taps used for sampling treated waters that require microbiological analysis, should be disinfected before being sampled. There are exceptions to this practice, however, when for example, tests for protozoan parasites need to be carried out, or when other circumstances prevail where consumer complaints are to be investigated. In these cases, samples that are taken before the disinfection process is carried out may provide useful information on the quality of water sampled, or the condition of the tap and /or associated pipe work.

5.2.1 Disinfecting taps using chlorine based compounds

Sodium hypochlorite solution and other chlorine-generating solutions (for example dichloroisocyanurate) are generally used for disinfection. These solutions are highly corrosive, and should be handled with care. If these solutions come in contact with skin, the area should be washed immediately with copious amounts of cold or tepid water. A disinfectant solution comprising 1 % available chlorine (equivalent to 10000 mg/l as chlorine) has been found suitable for disinfecting taps. Suitably labelled "wash bottles" may be used to store this solution, but these solutions should, ideally, be prepared on the day of use from either commercially available stock solutions (i.e. dilutions of 10 - 14 % solution of sodium hypochlorite) or 'chlorine tablets' (composed of for example 50 % sodium dichloro-1,3,5-triazinetrione).

Taps can be disinfected in several ways. One way, for example, is to disinfect a tap using 1 % available chlorine solution. Where practicable, all external fittings should be removed from the tap. Any accumulated deposits should then be removed (in an appropriate manner) from the tap. For example, grease and slime, etc can be removed using a proprietary *iso*-propyl alcohol wipe. The tap should then be turned on and water allowed to run to waste, at a uniform flow rate, in order to remove any debris, sediment and/or biofilm contained within the tap and associated pipe work, and to clear any standing water in the pipe work. Depending on individual circumstances, this flushing process may take several minutes or longer. It may be helpful at this stage to measure the temperature of the water issuing from the tap until a stable value is obtained. The residual chlorine concentrations should then be determined in the water.

When the tap and associated pipe work are free of debris, sediment and/or biofilm, and where appropriate a stable temperature value is obtained, and the chlorine tests are completed, the tap should be turned off. Disinfection of the tap can then proceed using a wash bottle, or similar device, filled with the disinfectant solution. The nozzle of the wash bottle should be inserted, as far as possible, into the spout of the tap and disinfectant solution injected into the tap until the solution runs out of the tap. Disinfectant solution should also be sprayed onto the exterior part of the tap, particularly around the spout. Care should be taken to ensure that the disinfectant solution does not spray out from the tap onto areas where it might result in damage to fixtures and fittings, or cause personal injury. Once the tap has been sprayed with disinfectant solution, it should be left for two to three minutes in order to allow the disinfection process to take place.

Once the disinfection time has elapsed, the tap should be turned on gently. Care should be taken with the exterior part of the tap as this may still contain traces of disinfectant. The water is then allowed to run to waste at a uniform flow rate to ensure that all the disinfectant solution

is removed from the inside of the tap. Depending on individual circumstances, this flushing process may take several minutes or longer. The residual chlorine concentrations should be determined again to ensure that levels have returned to those levels previously determined. Failure to flush the tap adequately will lead to elevated levels of residual chlorine concentrations. This in turn will lead to a sample being collected that is not representative and contains excess disinfectant solution.

In certain circumstances, and recognising that modern plastic anti-splash devices can harbour bacteria and fungi, it may be beneficial to repeat the disinfection process, i.e. disinfect the tap twice. It has been shown that, in some cases, disinfecting the tap, allowing water to run to waste and disinfecting the tap again, can enhance the decontamination process.

5.2.2 Disinfecting metal taps by flaming

Except for those metal taps fitted with non removable plastic anti-splash devices, disinfecting metal taps may be carried out by flaming them. It may be however, that consumers may not favour this approach, and in these circumstances, sampling staff should use an alternative approach.

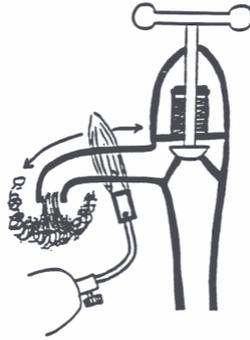
Where practicable, all external fittings should be removed from the tap. Any accumulated deposits should then be removed from the tap. For example, grease and slime, etc can be removed using a proprietary *iso*-propyl alcohol wipe. The tap should then be turned on and water allowed to run to waste, at a uniform flow rate, in order to remove any debris, sediment and/or biofilm contained within the tap and associated pipe work, and to clear any standing water in the pipe work. Depending on individual circumstances, this flushing process may take several minutes or longer. At this stage, measure the temperature of the water issuing from the tap until a stable value is obtained. When flushing is complete gently turn the tap off.

To disinfect a metal tap by flaming, a small proprietary propane or butane burner can be used. The burner should produce a tight, controllable flame. When the burner is being used, care should be taken, and the flame directed away from flammable or heat-sensitive items such as curtains and papers.

Methylated spirits should not be used as burners using this solvent are more difficult to control. In addition, the temperature of the flame is not sufficient to ensure adequate heat transfer through the metal tap to disinfect it.

With the tap turned off, start flaming it at the nozzle, gently moving the flame backwards and forwards over the spout of the tap, until steam and boiling water issues from the nozzle. See Figure 2. Care should be taken to ensure that steam and hot water, which may spurt out of the nozzle during flaming, does not cause damage to fixtures and fittings, or personal injury. If the design of the tap is such that water drains out of the tap when it is turned off, the full length of the spout should be heated such that, when the tap is turned back on, the first issue of water boils momentarily.

Figure 2 Example of flaming a metal tap



After flaming the tap and prior to collecting the sample, the tap should be turned on and water allowed to run to waste, at a uniform flow rate, until pipe work is cleared of standing water. Measure the temperature of the water issuing from the tap and continue flushing the water until the water temperature reaches that value measured before flaming. Depending on individual circumstances, this flushing process may take several minutes or longer.

5.3 Dip sampling

Dip samples (i.e. samples obtained by immersing an open container or device into a body of water) may occasionally need to be taken, for example for investigational purposes. Dip sampling should not be used for taking samples for regulatory drinking water compliance purposes.

When a dip sample is taken for microbiological analysis, a sterile container or device should be used. Sterile dip apparatus and sample containers, for example wide-mouthed 500 ml sample bottles, can be prepared and these are usually attached to a wire or chain of sufficient length. The wire or chain can be attached either directly to a sample container or via a bottle cage of sufficient weight. The sample bottle, with cage and wire or chain, can be wrapped in suitable material and autoclaved with, if necessary, the lid being wrapped separately. When required, the wrapping material should be removed from the bottle, which is then dipped into the water until the bottle is filled and then removed from the water. The lid (if wrapped separately) is then unwrapped and carefully placed on the bottle.

To prevent cross contamination from one sample to another, the sampling device should be thoroughly disinfected between each sampling occasion, or a fresh device, sterilised by autoclaving, used. If the device is disinfected using chlorine-based reagents, it should be assured that disinfectant does not gain access to the water being sampled. It may be appropriate to determine free and combined residual chlorine levels in the water.

Commercially available sterilised disposable dip samplers, i.e. dip samplers that are used only once and are mounted onto short rod handles may be suitable for use when taking dip samples from small tanks or similar sample points.

Alternative apparatus may also be used. For example, for taking dip samples from service reservoirs a jug attached to a chain, each made of metal, may be used. The jug and chain

should be stored in a clean plastic bag between each sampling occasion. Before use, the jug should be removed from the bag, flame-sterilised over its entire surface, and allowed to cool. The chain should be flamed and allowed to cool, as the jug is lowered into the water in the service reservoir. Care should be taken to ensure that heat transfer from the jug and chain does not affect the microbiological quality of the water being sampled.

When sufficient water has been collected (this may take more than one dipping operation) the jug should be retrieved and water carefully decanted into one or more sample containers. Care should be taken to avoid splashing. When the sample container is full, leaving a small air gap or head space, the container should be sealed for submission to the laboratory for analysis.

When samples are required for bacteriological, physical and chemical determinations, separate containers should be used. Sterile containers are required for bacteriological samples. Containers for physical and chemical determinations need not be sterile.

5.4 Bacteriological swabs

Additional information on the microbiological quality of the water and condition of a tap can often be obtained by taking swab samples from the inside of the tap and around the nozzle. This can be difficult with certain types of taps, where the design of the tap prevents or inhibits the insertion of the swab into the tap. Depending on the reason for taking the swab sample, it can be taken either before or after the tap has been disinfected. For example, results from a swab sample taken before disinfecting the tap may be used to investigate whether the tap or water is contaminated with bacteria and compared with results from a swab sample taken after the tap has been correctly disinfected. In addition, swab samples taken from taps that have been disinfected can be used to demonstrate the efficacy of the disinfection procedure.

To take a swab sample, a sterile swab should be removed from its container and the tip inserted into the nozzle of the tap. Care should be taken to ensure no other surfaces come in contact with the tip of the swab. The swab is then “rubbed around”, i.e. moved in a backwards and forwards, and up and down motion, as much as possible, on the inside surface of the tap, or tap insert. The swab should then be carefully replaced in its container, again ensuring no other surfaces come in contact with the tip of the swab. Alternatively, swabs coupled with integrated transport medium may also be used. These are available commercially.

The container should then be labelled with appropriate details and kept cool by, for example placing it in an insulated cool box or refrigerator for storing and transport to the laboratory, (see section 7).

6 Microbiological sampling locations

6.1 Raw waters and partially treated waters

The analysis of raw waters used for drinking water abstraction is a regulatory requirement for drinking water compliance purposes⁽⁴⁾. Regular sampling and analysis of raw waters can provide essential information relating to its treatment requirements and the indication that potential hazards exist within the catchment area. Samples taken at locations within the treatment process may, similarly, provide useful operational information regarding the performance of the treatment at various stages within the treatment works.

6.1.1 Surface waters

In order to collect a representative sample of raw, or partially treated, water, the mixing characteristics of the water, or the presence or absence of sediments (and how these sediments may be disturbed) should be taken into consideration in the selection of appropriate sampling points. In addition, the possibility of “dead water” areas, i.e. areas of water which are motionless and remain still or stagnate, should also be taken into account. Dip samples (see section 5.3) taken for algae or zooplankton analysis, may also be required depending on the information required. These samples may be taken from the surface of the water or at pre-determined depths within the water.

Ideally, samples of raw and partially treated water requiring microbiological and chemical analysis at various stages of the treatment process should be supplied through dedicated taps and sample lines made from approved materials. Sample lines should be as short as practicable and capable of being flushed prior to sampling. It should be possible to sample the water without using pumps or apparatus for disinfection, halting the flow of water or interrupting the flow of water to any monitoring or control equipment. The actual location of sampling points will depend on the circumstances at each treatment works. Occasionally, it may be necessary to take dip samples. If partially treated water is sampled at process points, stringent precautions should be taken to avoid contamination.

Where it is necessary to take samples directly from rivers and streams, sites should be selected carefully. Health and safety implications should be considered, and those areas, for example discharge points, where marked water quality changes might occur should be avoided. Where available, convenient bridges or gantries provide more suitable locations than the banks of rivers or reservoirs.

6.1.2 Ground waters

When samples are taken from boreholes or wells, they should be representative of the water contained in the borehole or well, and be taken from dedicated taps that can be disinfected. The sampling of both raw and treated ground waters is usually required.

The application of a pump and the designation of the depth at which a sample is taken should be determined from knowledge of local hydro-geological considerations and the site from which the sample is to be taken. The influence of casings and locations where biofilms may form should also be considered. Where pumping is intermittent or variable, knowledge of

pumping regimes may be essential to ensure a representative sample is taken, and that the subsequent interpretation of results is correct. In addition, the effect of hydraulic shock when pumps are turned on or off may need to be considered as the effects caused may influence certain water quality parameter determinations.

Different types of ground waters, for example springs, which may or may not flow naturally out of the ground, may also need to be sampled. These sources may be sampled from a collection tank, and samples may be obtained by dip sampling or from a tap.

Further guidance on the sampling of ground water is available elsewhere⁽¹⁵⁾.

6.2 Water treatment works

For drinking water compliance monitoring purposes, the sample of water leaving the water treatment works should be representative of the final water being produced and should be taken from a discrete and dedicated sampling point tap. Ideally, these taps and associated sample lines should not be connected to any other equipment, or used for other purposes. Figure 3 shows example sample lines and taps that are incorrectly located.

Figure 3 Examples of incorrect tap locations (dedicated supply not provided)



Sampling points at water treatment works should be fitted with appropriate metal sampling taps which conform to national standards⁽¹⁶⁾. These taps should not be fitted with attachments or inserts, and should be clean, free from extraneous matter, for example slime, grease, cleansing or disinfection agents, which may affect the microbiological quality of the water being sampled. The neck section of swan neck taps can harbour biofilm growth. Hence, appropriate cleaning (for example, disinfection and/or flame sterilisation) should be considered in order to ensure potential biofilm growth does not impact on samples when these types of taps are used. Plastic or mixer taps are not suitable for sampling water required for microbiological analysis at water treatment works. Sample taps should be provided on each outlet main of the water treatment works and be adequately labelled with, for example the location and required flushing time (if appropriate). Sample taps may be left continuously running. Microbiological samples may be taken from such taps without

disinfecting them, provided there are no leaks from the tap and the flow of water does not change during the sampling process.

For drinking water compliance purposes, the associated pipe-work to the tap used for collecting samples should be as short as practicable, made of approved materials (regulation 31⁽⁴⁾) and be maintained in good condition. At these taps, there should be adequate pressure and flow of water to enable samples to be taken whenever the water treatment works is operational or in use. Where the water pressure at the tap is known to fluctuate, or be unacceptably low, alternative sampling arrangements should be made.

Ideally, the sample point should be situated so as to minimise the occurrence of contamination, for example caused by splashing, and there should be adequate drainage for water to run to waste to prevent the accumulation of water at the sampling point.

If a sample of water is taken and analysed and the results show that the water quality is not acceptable, for example there is an infringement of a regulatory requirement, it is usually necessary not only to take additional samples from the original sample point but also to consider other locations within the water treatment works, to increase the volume of sample collected and/or take samples from within the distribution system.

Further guidance on sampling of drinking water from treatment works and piped distribution systems can be found elsewhere⁽¹⁷⁾. Guidance on sampling procedures for protozoan parasites (for example, *Cryptosporidium* oocysts and *Giardia* cysts) and viruses can be found in section 8 and elsewhere^(12, 18).

6.3 Service reservoirs and water towers

Service reservoirs and water towers are, potentially, vulnerable parts of any distribution system where microbiological ingress can occur. It is important, therefore, that any sample taken at these locations is representative of the water leaving the reservoir or tower. Thus, sampling regimes should take into account such factors as flow patterns and joint or common inlet or outlet arrangements. For regulatory drinking water compliance purposes, microbiological samples should be taken from dedicated and discrete taps. All service reservoir compartments should be fitted with individual sampling taps in order that the microbiological quality of water within each compartment can be determined. This also facilitates investigatory studies where microbiological failures are observed in reservoirs and towers. In addition, sample lines should not be connected to other equipment, and the taps should not be used for other purposes. Further advice on the location of compliance sampling points at service reservoirs is provided elsewhere⁽⁴⁾.

Sample taps are often situated in specialised lockable cabinets, for example metal cabinets, see Figure 4. This helps to prevent vandalism to the tap and its mis-use, and also in isolated locations, prevents damage caused by freezing.

Figure 4 Example of a lockable sample cabinet



The actual tap may be for example a “Harris” type tap, see Figure 5. This type of tap is made of a chrome plated gun metal construction, possessing a tapered spout. When not in use the spout is kept clean and further protected by a cover. To prevent its loss or misplacement, this cover is secured to the tap with a chain.

Figure 5 Example of a Harris type tap



All service reservoir compartments should be fitted with individual sampling taps in order that the microbiological quality of water within each compartment can be determined.

Where it is not possible or practicable to provide a dedicated sampling tap on-site, an alternative tap should be provided for each reservoir or tower outlet at the nearest practicable and most suitable location. This location should be positioned before the premises of the first customer

Under adverse weather conditions, or other extreme prevailing circumstances, service reservoirs located in remote places may become inaccessible. In these situations it may be impossible to take a sample from the reservoir. Even while these conditions and circumstances prevail, it is still important to continue to monitor the quality of water supplied from such sites. Where the service reservoir cannot be sampled, and as a short-term measure, a sample should be taken from the premises of the first customer supplied by the service reservoir.

The use of sample pumps for taking bacteriological samples should, generally, be discouraged. However, for certain service reservoirs, their use may offer the only practical means of obtaining a sample. The sample pumps used should be chosen to ensure there is minimal risk of biofilms forming within the internal structures of the pump. To prevent the formation of biofilms, reduce pump wear and reduce stagnation of water in the sample line the use of timed, automatic flushing systems may prove helpful. If used, sample pumps should be well maintained and tested to show that satisfactory bacteriological results are produced.

Sampling taps at service reservoirs and towers should be of appropriate materials that conform to national standards⁽¹⁶⁾. These taps should not be fitted with attachments or inserts and should be clean, free from extraneous matter such as slime or grease, cleansing or disinfection agents, etc that may affect the microbiological quality of the sample being taken.

Sample taps can, in certain situations, harbour biofilms. Hence, appropriate cleaning (for example, chlorine disinfection and/or flame sterilisation) should be undertaken as for customer taps in order to ensure biofilm growth does not impact on the quality of the water being sampled. (See sections 5.2 and 5.3). Plastic or mixer taps should not be installed at service reservoir sampling points. Sample taps should be provided at each outlet main of the service reservoir and should be used exclusively for regulatory drinking water compliance purposes. In addition, these taps should be labelled with, for example, the exact location and where necessary the required flushing time.

Dip samples should not be taken at service reservoirs or water towers unless it is unavoidable. This is to prevent any potential risk of contaminating the main bulk of the water within the reservoir or tower whilst sampling the water. However, when, for example, operational investigations need to be carried out, or when a compartment is isolated, dip sampling may offer the only means of taking a sample. Under these situations, the procedures described in section 5.3 should be strictly adhered to in order to minimise the risk of extraneous material gaining access to the reservoir water.

6.4 Domestic properties

Taps at domestic properties should, ideally, be in good repair and should supply water from a pipe connected directly to the water main. The tap within the domestic property from which a sample is to be collected for regulatory drinking water compliance purposes might, normally, be a kitchen tap, or a tap that is regularly used in connection with drinking and cooking purposes⁽¹⁹⁾. These taps should be connected directly to the water main and should be capable of being disinfected. Taps in other rooms, for example within bathrooms and/or toilets, or external taps might also need to be used, especially for investigational purposes (for example following customer complaints). However, taps supplied from a cistern or storage tank, or via an in-line filter or water softener may not be suitable.

Unless there are exceptional or local circumstances or the safety of sampling staff are prime considerations that make it impracticable, samples taken for regulatory drinking water compliance monitoring purposes should be taken from randomly selected properties, based on a pre-scheduled process. Where pre-scheduled properties cannot be accessed, an alternative property, located nearby, should be selected, and appropriate records subsequently amended. Details should also be recorded as to why water from the original pre-selected property was not sampled.

The procedures used to disinfect taps will depend on the type of tap to be sampled. Certain taps may contain anti-splash devices or may be made of materials which render them difficult, or impractical, to disinfect. Ideally, sample taps should be clean, free from extraneous matter such as slime, grease, cleansing or disinfecting agents and attachments which might affect the microbiological quality of the water being sampled. Taps that leak between the spindle and the gland when the tap is turned on should not be used. Uni-flow and other mixer taps, see Figure 6, where the hot and cold water mix together within or at the spout of the tap might not provide a representative sample of the cold water supply. In these cases, guidance should be sought as to the most appropriate procedure to be used for disinfecting these types of taps. See section 5.2.

Figure 6 Example of a mixer tap



6.5 High-rise buildings

It may often be difficult to determine whether a tap within a property in a high-rise building is supplied directly from the mains or via a storage tank. Whenever possible, advice and guidance should be sought to identify the source of supply to a tap being considered for sampling before it is undertaken. Samples taken for regulatory drinking water compliance purposes should only be taken from taps that supply water from a pipe connected directly to the main.

6.6 Public buildings

Generally, public buildings, including restaurants, schools and hospitals are buildings where drinking water is made available to members of the public. The microbiological quality of drinking water (supplied at taps within public buildings for drinking water purposes or used for food preparation) should satisfy minimum regulatory standards. Taps within public buildings intended to be sampled for drinking water compliance purposes should, ideally, be labelled as providing water for drinking.

In the UK it is a regulatory requirement for a water company to take a proportion of its drinking water compliance samples from public buildings⁽⁴⁾. Whilst it is normally regarded as good practice to disinfect taps when taking microbiological samples (see section 5.2) it may be appropriate in public buildings to consider taking samples without disinfecting the tap. This is likely to lead to the situation whereby the quality of the water supplied is representative of the quality experienced by consumers. The disinfection of taps with chlorine, in public buildings, is interpreted as an act of maintenance. Where such a tap would normally be turned on and water allowed to run to waste, for example into a sink, before drawing water for drinking, flushing the tap (see section 5.2) should still be undertaken before sampling. Further guidance is provided elsewhere⁽²⁰⁾. The purpose of sampling and the individual circumstances surrounding this event should be taken into consideration. In some cases, for example for investigational purposes, it may be appropriate to take samples before and after disinfecting a particular tap within a public building.

As with high-rise buildings, it may be difficult in public buildings to ascertain whether a tap is supplied directly from the mains or via a storage tank. When samples are taken from taps within public buildings, and particularly when this is for regulatory drinking water compliance purposes, appropriate details should be recorded, including for example, the type of building sampled, the exact location of the tap used, and any information or uncertainty about the nature of the supply of water to the tap.

6.7 Drinking water fountains

Drinking water fountains may be used as a means of providing drinking water to members of the public. These fountains may be located within buildings, such as schools or offices, or outside, in recreational areas open to the public, for example parks and sports arenas.

It is often difficult to maintain drinking water fountains in a clean and hygienic condition. The design and installation of drinking water fountains, as well as the application of effective cleaning and maintenance regimes are essential features for maintaining the microbiological quality of the water supplied. Sampling and analysis of the water can play an important role in demonstrating its quality.

Depending on the design of the drinking water fountain it can be difficult to obtain a representative sample. As with taps in public buildings it may be appropriate to sample drinking water fountains after flushing has taken place (see section 5.2) omitting the disinfection stage. The purpose of sampling and the individual circumstances surrounding the event should be taken into consideration. In certain cases, for example for investigational purposes, and it may be appropriate to take samples before and after disinfecting the

fountain. For comparison purposes, if the drinking water fountain is supplied from a distribution network it is prudent at the same time, to sample from a nearby property.

When a drinking water fountain is sampled, appropriate details such as the exact location and description of the fountain should be recorded together with any observations about its condition.

6.8 Drinks-vending machines

Vending and dispensing machines utilise a variety of equipment, many of which require a supply of wholesome water. Such equipment may involve the supply of hot and cold water for making beverages prepared from, for example powdered ingredients or syrups. Whilst such vending machines should be permanently connected, i.e. “plumbed-in”, to a potable water supply, older and smaller machines may be hand-filled. Additional guidance and details on all aspects of vending and dispensing operations are given elsewhere⁽²¹⁾. Sampling arrangements will vary according to the design of the machine. Most “plumbed-in” machines do not usually possess facilities for flushing water to waste prior to sampling. In these cases, when these machines are first used during each day, they are likely to draw water (from the supply pipe-work) that has been left standing for some time, for example overnight. It may, therefore, be inappropriate to sample the water entering these machines until after a procedure has been devised that allows water to run to waste prior to sampling.

If the quality of the water being supplied to the vending machine is to be compared with the quality of water emanating from the vending machine, appropriate samples should be taken. For example, should the water supplied to the vending machine be water

- (i) that has been allowed to stand for long periods in the supply pipe work,
- (ii) from a storage tank, or
- (iii) from a frequently used tap located near the vending machine.

The water emanating from the vending machine should be water supplied from the cup station of the vending machine.

Drinking water fountains, and water dispensers utilising an attached container, frequently make use of water coolers⁽²²⁾. In these situations, samples should be taken directly from the tap attached to these units. Samples should be collected in sterile containers.

Where water from vending machines is to be tested for compliance with the drinking water standards prescribed in the regulations⁽⁴⁾, tests should include the determination of *E. coli*, coliform bacteria and colony counts. If an unusual result is found, it may be necessary to clean or replace the dispensing point equipment or the cup station, before further samples are taken.

6.9 Aircraft, ships and trains etc

Storage tanks on trains, ships and aircraft may be filled with water from a piped main either via a standpipe or hose, or from a bowser filled from a standpipe. The standpipe and hoses should be protected from contamination and flushed before filling the bowser or storage tank. The tanks and bowsers should be drained, cleaned if necessary and disinfected at frequent

intervals. Care should also be taken to protect the integrity of the system and to avoid contaminating the system or making inappropriate cross-connections. Any on-board treatment systems should be adequately maintained.

A suitable tap should be available for sampling the water within each aircraft, ship or train, etc. It is important that staff sampling water in these locations possess an understanding of the arrangements surrounding the supply of water to the tap used, or are provided with necessary details or information. The procedures described for domestic properties should be followed, see section 6.4.

To reduce potential hazards and for safety reasons, taps in these locations should not be flame disinfected. Occasionally, dip samples may need to be taken, for example from storage tanks, and if so, the procedures described in section 5.3 should be used.

6.10 Hydrants

It is often necessary to take samples, for example for investigational or operational purposes, from locations such as new, renovated or repaired mains. In these cases, the sample may be taken directly from the mains supply by means of a standpipe, attached to a hydrant or meter box. Samples for regulatory drinking water compliance purposes should not be taken directly from hydrants.

In order to take a sample from a hydrant, the hydrant box lid should be removed and any debris present in the area surrounding or in proximity to the connection (the hydrant bowl) should be cleared away and disposed of appropriately. Sampling staff should be aware of potential risks and dangers associated with the nature and type of debris that may accumulate in hydrant boxes (for example the presence of sharp or dangerous objects). Staff should, therefore, take appropriate precautions and wear personal protective equipment, where required.

Any water present in the hydrant box should be removed, for example by bailing it out using an appropriate container. If all the water cannot be removed, the amount remaining should be to an acceptable level, for example several centimetres below the bottom of the screw threads on the hydrant connection, such that it does not cause contamination to the water being sampled. The hydrant bowl cover (if present) should be unscrewed. The hydrant valve should then be partially opened to allow water to run to waste. This water should be sufficient (for example, approximately 5 litres) to flush away all the debris from the hydrant bowl and should be allowed to gently cascade directly into the hydrant box. If the water cannot drain away, it should be removed and the amount remaining should be to an acceptable level that does not cause contamination to the water being sampled, for example several centimetres below the bottom of the screw threads on the hydrant connection.

Before use, the standpipe and tap should be clean, and stored and transported in a clean plastic bag. Standpipes used for microbiological sampling should not be used for other purposes. The standpipe should be removed from the plastic bag and connected to the hydrant bowl. The standpipe tap and the hydrant valve should then be smoothly opened to produce a gentle flow of water. Rapid valve or tap movement should be avoided, as this might cause turbulence to occur in the mains supply resulting in sediment becoming dislodged. The standpipe should be flushed to remove any sediment that may be present in the mains, until

the water is clear, for example for a minimum of 5 minutes. The residual chlorine concentration of the water should then be determined.

If net-samples are to be taken (for example for zooplankton determinations) they should be taken at this stage by allowing water to run through a suitable net. The hydrant valve should then be closed and the standpipe removed. Approximately 200 ml of water should be bailed out from the hydrant bowl and 100 ml of chlorine solution (see section 5.2.1) carefully added to the hydrant bowl. The standpipe should then be placed back into position, and the hydrant valve opened gently until a flow of chlorinated water just begins to discharge from the tap. The hydrant valve should then be closed and the standpipe left to stand to ensure adequate chlorination to occur, for example a minimum of 5 minutes. Additionally, the standpipe tap should be flame sterilised (as described in section 5.2.2).

The hydrant should then be re-opened and water in the standpipe flushed through the tap for approximately 1 minute. The chlorine concentration should be measured to ensure that the disinfectant has been flushed out. A sample of the water should then be taken in the same manner as described in section 5.

The hydrant valve should then be closed and the standpipe removed. Finally, the hydrant cap and box lid should be replaced.

Different types of standpipes are available. A two-inch standpipe is generally used for flushing, and a bib tap half-inch standpipe (with a permanently attached tap) is used for sampling. Alternatively, a 'combination' standpipe, see Figure 7, can be used. This comprises a two-inch standpipe with a side tap, which can be used for direct sampling after an appropriate period of flushing. Standpipes with non-return valves may harbour a build up of debris and/or biofilm, and sampling downstream of such valves may give rise to samples that are not representative of the water being sampled. A combination standpipe (where the tap is upstream of the non-return valve) may be more suitable.

Figure 7 Example of a combination standpipe



6.11 Bowsers and tankers

All bowsers and tankers deployed for example during emergencies or mains renovation work should be sampled at least every 48 hours when they are deployed. All taps and hatches, etc should be checked to ensure they have not been damaged or vandalised and that they are secure prior to sampling. The concentration of free and combined residual chlorine in the water should be determined prior to sampling. The tap should be turned on and run to waste in order to displace any water present in the tap. This usually takes about 10 seconds. The tap should then be disinfected (sections 5.2.1 and 5.2.2), and a sample taken, as described in section 5. Some bowsers and static tanks may have plastic taps, and if so, disinfectant solution may be more appropriate for disinfection.

6.12 Bottled water

UK Legislation⁽⁷⁾ requires that any bottled water supplied instead of mains water, for example during emergencies or mains renovation work, should be of an equivalent quality to the mains water usually supplied to the area. Bottles of water representative of the bottled water supplied should be selected at random from each batch of bottled water supplied, on receipt of the bottled water by the water company, and at intervals during storage and use. These randomly chosen bottles of water should be submitted to the laboratory for microbiological analysis. The analysis should include those parameters normally determined for the mains water. If bottles of water are deployed for periods in excess of 48 hours, random samples should be chosen and analysed at least every 48 hours.

7 Transport and storage of samples

The microbiological characteristics of a sample can change significantly when stored, even for relatively short periods of time. Samples should, therefore, be analysed as soon as practicable on the day of collection. Once taken, microbiological samples should, at all times, be stored in the dark in the temperature range of 5 ± 3 °C. In any event, the analysis should commence within 24 hours of the sample being taken.

Insulated cool boxes, small refrigerators or refrigerated vehicles are suitable for storing and transporting samples to the laboratory. Insulated cool boxes and small refrigerators used for the transport of microbiological samples should be used exclusively for this purpose and should not contain samples of both raw and treated waters. Where practicable, the storage temperature should be monitored and recorded. Insulated cool boxes and small refrigerators should be kept clean and dry, and should be regularly disinfected. Vehicle storage racks or boxes should be treated similarly.

A number of studies^(23, 24) have been undertaken to assess the effects of storage and transport on microbial levels in water samples. The effect of storage time between sampling and commencement of analysis is influenced by many factors including the ambient water chemistry of the sample, the number and type of organisms present and their physiological state after exposure to disinfectants. Clearly, the longer the delay between sampling and commencement of analysis, the more likely that microbiological changes will occur within the sample.

Further guidance on the preservation and handling of water samples can be obtained elsewhere⁽²⁵⁾.

8 Sampling for protozoan parasites, viruses and other special investigations

Guidance is provided elsewhere in this series⁽¹²⁾ on the sampling and analysis of *Cryptosporidium*.

Samples for virological determinations are usually taken in 10 litre containers, which are then submitted directly to the laboratory. Further guidance is described elsewhere⁽¹⁸⁾.

Samples requiring *Legionella* analysis are usually taken in 1 litre containers, which are then submitted directly to the laboratory. Further guidance is described elsewhere^(26, 27).

Appropriate information and documentation (see section 4.2) should accompany all samples, filters and containers, etc submitted to the laboratory for analysis. Where a sample requires an unusual analysis, advice should be sought from the laboratory undertaking the analysis.

9 References

1. ISO 5667-1: 2006 Water Quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques.
2. Standing Committee of Analysts - General Principles of Sampling Water and Associated Materials (second edition) 1996; *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency. ISBN 011752364X
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Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

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