



ENVIRONMENT AGENCY

**The Microbiology of Drinking Water (2002) - 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 contains details of the practices and procedures that should be adopted for taking samples for microbiological analysis.

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

Part 1 - Water quality and public health

Part 3 - Practices and procedures for laboratories

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

Part 5 - A method for 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 7 - Methods for the enumeration of heterotrophic bacteria by pour and spread plate techniques

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

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

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

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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. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

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.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

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. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

- 1 General principles of sampling and accuracy of results
- 2 Microbiological methods
- 3 Empirical and physical methods
- 4 Metals and metalloids
- 5 General non-metallic substances
- 6 Organic impurities
- 7 Biological methods
- 8 Biodegradability and inhibition methods
- 9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods is available from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

January 2002

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 1999 (SI 1999/437). 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

Taking a sample in the right container in a correct manner and storing it appropriately until commencement of analysis is a vital part of any microbiological analysis. If the sample is not representative of the material under test, is contaminated during sampling or storage, or is incorrectly stored during transport, then the reported analytical result will be misleading, no matter how sophisticated or reliable the analytical method.

2 The sampling manual

Organisations undertaking water sampling should set out in a sampling manual details of procedures and precautions that should be adopted during sampling. This manual should be available to all sampling staff. As a minimum, the manual should include:

- (i) a description of all sample bottles and their uses;
- (ii) a full description of the sampling procedures to be used at each type of location including, where necessary, the order of sampling;
- (iii) advice on how to avoid accidental contamination of the sample during sampling; and
- (iv) advice on transporting samples to the laboratory.

3 Sample containers

Sample bottles should be made of suitable pre-sterilised disposable or autoclavable plastic, or good quality soda or borosilicate glass, and be free from toxic substances. Bottles should be used exclusively for microbiological sampling and for no other purpose and glass bottles should not be used for sampling purposes on food manufacturing premises. In addition, containers showing any signs of defect should not be used. The size of the bottle required depends on the number and type of tests to be carried out. A capacity of about 300 - 500 ml should be sufficient for most routine testing for indicator bacteria, but larger volumes will be necessary for the examination of protozoan parasites and for other special investigations. Sample bottles should always be clearly labelled to enable a laboratory to identify the contents.

3.1 Container preparation

Re-useable bottles and caps should be washed thoroughly with phosphate-free, non-toxic detergents, followed by thorough rinsing with distilled or deionised water and then allowed to drain. After use, bottles should be rinsed with distilled or deionised water. Most laboratory washing machines provide suitable washing cycles to accommodate this.

Treated water often contains traces of chlorine or chloramines. Hence, sufficient sodium thiosulphate should be added to all sample bottles for microbiological use to neutralise these substances and ensure that disinfection does not continue to occur within the sample bottle. At pH values normally occurring in water supplies, the addition of sodium thiosulphate

pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) at a concentration of 18 mg/l should neutralise up to 5 mg/l of free and combined residual chlorine. Sodium thiosulphate is reported to have no significant effect on *E. coli* or coliform bacteria in unchlorinated water during storage⁽¹⁾. The appropriate concentration of sodium thiosulphate pentahydrate should, therefore, be added to all microbiological sampling bottles before they are sterilised. This can be achieved by adding to the sample bottle an amount of a freshly prepared 1.8 % m/v solution of sodium thiosulphate pentahydrate (usually 0.1 ml) for each 100 ml of bottle capacity. Bottles should then be capped or stoppered, and where necessary (for example, bottles with glass-stoppers) the stopper covered with another suitable additional cover (for example, metal foil) before sterilisation. Periodically, after sterilisation, a check should be carried out to ensure the efficacy of the sodium thiosulphate (for example, by challenging the neutralising solution with a solution of chlorine, in distilled or deionised water at a concentration of 5 mg/l).

Sample bottles may comprise caps made of autoclavable plastic, or metal with silicone rubber liners. If bottles with ground glass stoppers are used, a thin strip of paper or foil should be inserted between the stopper and the neck of the bottle before sterilisation. This prevents the stopper from jamming in the neck of the bottle and the possibility of the glass from cracking following cooling. Before sterilisation, bottles should be marked with suitable indicator tape that is capable of showing whether bottles have undergone the correct sterilisation process.

Glass bottles can be sterilised either by autoclaving at 121 °C for 20 minutes, or by heating to 160 °C in a fan-assisted oven and holding at that temperature for 60 minutes. For each batch of bottles, the sterilisation cycle should be monitored using a calibrated temperature probe placed at an appropriate position within the batch being sterilised. This should show whether each batch of bottles has received appropriate heat treatment. Bottles should only be released for sampling when the sterilisation process has been shown to be satisfactory. Indicator tape is not sufficient for this purpose alone, but is a useful visual aid of heat treatment, once a batch of bottles has been shown to be satisfactory. Sterilising equipment should be serviced at regular intervals and calibrated in terms of cycle-time, pressure and temperature performance according to the manufacturer's instructions.

Sterile bottles should be marked with a suitable batch code and the date of expiry, the date of expiry representing the last date by which the sample bottle should be used. Bottles should then be stored in a clean, dry location provided only for that purpose. Ideally, bottles should be capable of showing whether they have been tampered with. Systems for proper stock control should be documented and carried out to ensure that sterilisation, storage and use before the date of expiry are maintained. Particular attention should be paid to ensure appropriate sample bottle storage and stock control in sampling vans and at remote locations. Sample bottles should normally be used within three months of sterilisation. Any bottles that are not used before the date of expiry should be removed from storage, and washed and re-sterilised.

Where disposable, pre-sterilised bottles are used, they should be capable of showing whether they have been tampered with and be supplied with adequate records of their sterility. In addition, they should be used within the date of expiry specified by the manufacturer.

3.2 Container labels and sampling records

All sample bottles should be adequately labelled. Self-adhesive labels that are easily removed after use, prior to washing, are suitable for this purpose. Labels may be pre-printed or bar-coded, and information entered thereon or subsequently written upon should be in permanent or indelible ink. The information provided should clearly identify the sample and the purpose for which it was taken. This information should also be available on a sampling worksheet, record or log. The pre-printed or bar-coded label should provide, for example, the following information:

- (i) a 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); and
- (vi) the identity of the person taking the sample.

If the above information is not entered on a sample bottle label then the label should have a unique number, code or bar-code whereby the required information can be referenced to a worksheet or sampling record. Any additional information should be recorded at the time of sampling. For treated water and water within distribution systems, the residual chlorine concentration should always be recorded. Additional information may be of value when the results of microbiological analyses are interpreted, and this may be provided on the worksheet or sampling record. Such information might include comments on the sample tap and whether it was disinfected, details of the aesthetic quality of the water and weather conditions, or unusual features observed or encountered during sampling. The sampling point should always be identified in sufficient detail to enable a repeat sample to be taken from the same location.

4 Sampling of raw water and from treatment processes

The analysis of raw water is not a regulatory requirement for drinking water compliance purposes. However, regular sampling and analysis of raw water can provide information that is important, in terms of assessing adequate treatment, and for investigating water treatment problems. Samples taken from points within the treatment process may, similarly, provide useful operational information.

Raw water samples should be representative of the water quality, and samples from the treatment processes should be representative of the treated water at that point. This means that mixing characteristics, the presence or disturbance of sediments, and possible “dead water” areas should be taken into account in the selection of sampling points. Also, the safety of sampling staff should be a paramount consideration. Ideally, samples of raw water and water at each stage of the treatment process should be supplied through dedicated sample lines made from approved materials, and should be as short as practicable and capable of

being flushed prior to sampling. It should be possible to sample without switching off pumps or apparatus used for disinfection. The actual location of sampling points will depend on the circumstances of each treatment works. Occasionally, it may be necessary to take dip samples. If so, stringent precautions should be taken to avoid contamination (see section 6.9).

When samples from boreholes or wells are taken, these should be taken from taps that are capable of being disinfected and that are fitted to the rising main before the water passes into any reservoir or cistern. The depth at which a sample is taken should be determined from local hydrogeological considerations and site knowledge. The influence of casings where biofilms may form should also be considered.

5 Sampling of treated water

5.1 Water leaving water treatment works

The sample should be representative of the final water and be taken from a point following satisfactory completion of all treatment and contact with disinfectant.

Sampling points at treatment works should be fitted with appropriate metal sampling taps which conform with national standards⁽²⁾. No attachments or inserts should be fitted to these taps. Taps should be clean, free from slime, grease, cleansing or disinfection agents and other extraneous matter, which may affect the microbiological sample. Swan neck taps can harbour growth of biofilm in the swan neck section and, thus, appropriate cleaning is required in order to ensure this does not impact on any samples taken. Plastic or mixer taps are not suitable for compliance microbiological sampling at treatment works. Sample taps should be provided on each outlet main, used exclusively for sampling, and labelled clearly with the location and flushing time. For operational reasons, sample taps may be left continuously running. Microbiological samples may be taken from these taps provided that there are no leaks from the spindle and the tap is well maintained.

The delivery pipe-work to the sample tap should be as short as practicable, of a suitable material and be maintained in good condition. There should be adequate water pressure to enable samples to be taken whenever the treatment works is operational or in use. Ideally, the sample point should be situated well above ground level in order to minimise the occurrence of contamination due to splashing. In addition, there should be adequate drainage to prevent the accumulation of water at the sampling point.

Sampling procedures for protozoan pathogens (for example, *Cryptosporidium* oocysts and *Giardia* cysts) and viruses are described elsewhere^(3, 4, 5).

5.2 Service reservoirs and water towers

Service reservoirs and water towers are potentially vulnerable parts of distribution systems where microbiological ingress can occur. It is important, therefore, that a sample is representative of the water leaving the reservoir or tower and sampling regimes should take into account such factors as flow patterns and joint or common inlet/outlet arrangements.

For regulatory drinking water compliance purposes, a microbiological sample should be taken from each service reservoir and water tower, even where the same site is a shared location.

Where a service reservoir or water tower has more than one compartment that share a common outlet main, a single sample from this outlet is acceptable for drinking water compliance sampling. If separate compartments feed separate outlet mains, each outlet should be sampled, unless the compartments are hydraulically connected. Where it is impossible to provide a tap on site, a tap should be provided for each outlet at the nearest practicable and most suitable point to the reservoir or tower. For example, if an outlet main is located very deep below ground level, or presents other sampling difficulties, a suitable, good quality tap at the nearest property fed directly off the outlet main might be appropriate.

Sampling points at service reservoirs and water towers should be fitted with appropriate metal sampling taps which conform with national standards⁽²⁾. No attachments or inserts should be fitted to these taps. Taps should be clean, free from slime, grease, cleansing or disinfection agents and other extraneous matter, which may affect the microbiological sample. Swan neck taps can harbour growth of biofilm in the swan neck section and, thus, appropriate cleaning is required in order to ensure this does not impact on any samples taken. Plastic or mixer taps are not suitable for compliance microbiological sampling at treatment works. Sample taps should be provided on each outlet main, used exclusively for sampling, and labelled clearly with the location and flushing time.

The taking of dip samples at service reservoirs and water towers is not advised because of the possibility of introducing contamination to the body of water within the reservoir or tower, within the sample, or both. Wherever possible, all compartments should be fitted with sampling taps. However, occasionally, for example, during operational investigations, dip sampling may be necessary and procedures (see section 6.9) should be described, and adhered to, to minimise the risk of extraneous material being introduced into the water supply.

5.3 Sampling taps at consumers' premises

From 1 January 2004, new regulations⁽⁶⁾ will require all drinking water samples taken for compliance monitoring to be taken from properties selected at random, unless exceptional, local circumstances or sampling staff safety considerations make this impracticable. In these circumstances, sampling requirements will be specified. Where randomly selected properties are not accessible, a nearby property should be selected, and appropriate records amended accordingly.

If a fixed sampling point has to be used, it should be a good quality customer's tap that can be thoroughly disinfected before sampling. When fixed points are used, they should be pre-determined and chosen in such a way that samples are representative of the water quality in the water supply zone. Fixed sampling points should only be changed where there is sufficient cause and preferably at the start of the calendar year. Under current legislation⁽⁷⁾, up to 50 % of the microbiological samples taken in a water supply zone may be taken from fixed sampling points.

The use of random sampling points may require the sampling of taps that might be considered unsuitable in controlled circumstances. The method for disinfection will depend on the type of tap; some 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 all attachments and from slime, grease, cleansing or disinfecting agents and other extraneous matter, which may affect the microbiological sample. Taps should be in good repair and

should supply water from a pipe connected directly to the main. Taps that leak, between the spindle and the gland, when the tap is turned on, should not be used. Uni-flow mixer taps (ie taps where the hot and cold water mix at the spout) should not be used but dual flow mixer taps may be used. In addition, taps supplied from a cistern or storage tank, or via an in-line filter or softener should not be used. Taps located on the outside of properties and connected directly to the main may be suitable, provided they are clean, not contaminated with garden detritus or chemicals, and are capable of being disinfected. These taps, however, cannot be used for regulatory drinking water compliance purposes, which require samples to be taken from taps normally used for the supply of water for human consumption⁽⁸⁾.

6 Sampling procedure

6.1 Order of sampling

Often, chemical sampling and physical and chemical testing will need to be carried out at the same time as the microbiological sample is taken and from the same sampling point. A correct order of sampling and associated on-site testing is therefore necessary to ensure that representative samples are taken and that the possibility of cross-contamination is minimised. An example is shown below:

- (i) Samples that need to be taken before flushing is carried out.
- (ii) Flush for a specified length of time.
- (iii) Sample, and carry out appropriate on-site physical and chemical tests (for example, chlorine residual, pH, temperature).
- (iv) Physical and chemical samples that need to be taken after flushing; also include biological samples, including invertebrate samples, if required.
- (v) Disinfect the tap (see sections 6.3 and 6.4).
- (vi) Bacteriological samples.

Depending upon individual circumstances, swab samples (see section 6.6), may be taken before or after flushing, or before or after disinfection of the tap depending whether the efficacy of the disinfection process is to be investigated.

6.2 Sampling from taps

Sample taps, used for sampling treated water intended for microbiological analysis, should be disinfected before being sampled. Exceptions to this practice include those circumstances where consumer complaints are investigated and where pre-disinfection samples may provide useful information.

6.3 Disinfection using sodium hypochlorite solution or similar solutions

Disinfection using sodium hypochlorite solution, or similar solutions, can be carried out on taps where flaming is not appropriate. Sodium hypochlorite solution and other chlorine

generating solutions are highly corrosive and should be handled with great care. If these solutions come into contact with skin, the area should be immediately washed with copious amounts of water. A 10 % solution of commercial sodium hypochlorite or other appropriate chlorine generating compound (for example, sodium dichloroisocyanurate) to give 1 % available chlorine should be used.

To disinfect a tap using sodium hypochlorite solution, or similar solutions, all external fittings should be removed from the tap. Accumulated deposits of grease and slime etc should be removed from the tap with, for example, a 2-propanol wipe or small brush. The tap should be turned on and left to run to waste, at a uniform rate, for a minimum of two minutes to clear standing water from the service pipe or sample line. For longer service pipes or sample lines, it may be more appropriate to monitor the temperature of the water until a stable reading is obtained. Disinfection can be carried out by swabbing the outside of the tap, and as much of the inside of the tap as is possible. Alternatively, a wash bottle, or similar device, filled with disinfection solution can be used to spray the outside the tap and inject the inside of the tap spout. Care should be taken to ensure that the disinfection solution does not spray out from the tap onto areas where it might result in damage or cause personal injury. Once the tap has been coated with disinfection solution, it should be left for two to three minutes to allow the chlorine to disinfect the tap. The outside of the tap should then be rinsed with water to ensure that there is no liquid residual disinfection solution left on the outside of the tap. The tap should be turned on and the water run to waste for a sufficient period of time to ensure that all the disinfecting solution is removed from the inside of the tap before taking the sample.

6.4 Disinfection by flaming

Disinfection by flaming can be carried out on metal taps, except those fitted with non-removable plastic anti-splash devices. Consumers may, however, not readily accept that their taps should be disinfected by flaming, and in these circumstances, sampling staff should offer alternative approaches, for example as described in section 6.3.

To disinfect a tap by flaming, a small proprietary propane or butane burner can be used. This should produce a tight, controllable flame. When the burner is being used, great care should be taken, and in particular, it should be stressed that flammable or heat sensitive items, such as curtains and papers, are removed from the immediate area. Methylated spirits should not be used as the flame is difficult to control and the temperature of the flame is not hot enough.

Prior to sampling, and in order to clear standing water from the service pipe or sample line, the tap is turned on and left to run at a uniform rate. This should be for a minimum of two minutes. For longer service pipes or sample lines, it may be more appropriate to monitor the temperature of the water until a stable reading is obtained. The tap is turned off and then flamed thoroughly, starting at the nozzle of the tap and working back to the body of the tap, until the water held in the spout boils. Care should be taken to ensure that hot water, which may spurt out of the tap during flaming, does not cause personal injury. If the design of the tap is such that the water drains out of the tap when it is turned off, the full length of spout of the tap should be heated such that, when the tap is turned back on, the first issue of water boils. After flaming, the tap is run to waste until the tap and water have cooled to the normal water temperature.

6.5 Taking the sample

The sample should always be taken in a sterile bottle. The bottle is 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 bottle. The sample bottle should never be rinsed out or the stopper or cap placed on any surface. From a gentle stream of water from the tap, the bottle is filled, leaving a small air gap. Any splashing should be avoided and the bottle should not be allowed to overflow. Whilst taking the sample, the flow-rate of the water leaving the tap should not be altered as to do so may cause biofilm, or other debris, to become dislodged within the system and to enter the sample bottle. When the sample has been taken the stopper or cap is replaced immediately, again taking care not to touch the top or neck of the bottle. The bottle is then placed in an insulated cool box or refrigerator for transport to the laboratory (see section 11). If, whilst taking the sample, accidental contamination is suspected, the sample should be discarded and a fresh sterile bottle used to take a new sample.

6.6 Bacteriological swabs

Additional information on the microbiological state of a tap can often be obtained by taking sterile swab samples of taps. It may be helpful to take swab samples at locations where customer complaints have been received, and at random addresses close to these locations. The swab sample should normally be taken before the tap is disinfected.

To take a swab sample, a sterile swab is removed from its container, and rubbed around as much of the inside surface of the tap as possible and then carefully replaced in its container. The container is then clearly labelled with appropriate details and placed in an insulated cool box or refrigerator for transport to the laboratory (see section 11).

6.7 Sampling from hydrants

When it is necessary to obtain a sample for operational or investigational purposes, such as sampling new, renovated or repaired mains, it is not always possible to obtain a sample from a consumer's tap that is connected directly to the main. In these cases, the sample may be taken directly from the main by means of a standpipe constructed specifically for the purpose and attached to a hydrant or meter box on a flow-through main. However, regulatory drinking water compliance samples must not be taken from hydrants or standpipes.

The hydrant box lid is removed and any debris cleared away from the area of the connection. Sampling staff should be warned of the dangers over the nature and type of debris that may accumulate in hydrant boxes and the need, therefore, to take appropriate precautions when removing such debris. Any water in the hydrant box should be bailed out to a level that is well below the bottom of the threads on the hydrant cover. The hydrant cover is then unscrewed and the hydrant valve opened a small amount to allow water to run to waste, sufficient to flush any debris from the hydrant. Usually, approximately five litres of water is sufficient to remove all debris.

The standpipe should be scrupulously clean and stored and transported in a clean plastic bag. The standpipe is unwrapped and connected to the hydrant. The standpipe tap and the hydrant valve are then opened smoothly. Any abrupt or rapid movement can cause turbulence to occur in the main resulting in sediment becoming dislodged. Water is then flushed through the

standpipe until it runs clear. If net-samples, for animals or similar biological samples, are required, they should be taken at this point. The water is then turned off.

The standpipe is removed and about 200 ml of water is bailed out from the hydrant bowl. Approximately 100 ml of 1 % solution of sodium hypochlorite or other appropriate chlorine generating compound (for example sodium dichloroisocyanurate) is added. The standpipe is then replaced and the valve opened slightly, until chlorinated water just discharges. The water is then turned off and left to stand for a sufficient time to ensure adequate chlorine disinfection. This usually takes 5 minutes. The water is turned on again and the hydrant flushed for about one minute. Without turning the water off, the chlorine content of the water is determined at intervals. When the final concentration is no higher than the chlorine level determined in the mains water, a sample of the water is taken in the same manner as described in section 6.5. The water is then turned off and the standpipe removed. The hydrant cap and box lid is then replaced.

If, during installation of the standpipe, it is suspected that the outside of the tap has been contaminated, it can be sterilised as described in sections 6.3 or 6.4.

6.8 Sampling from bowsers and tankers

All taps and hatches should be checked to ensure they are secure and are not damaged, and that the bower or tanker contains water. The concentration of residual chlorine in the water is determined. The tap is turned on, and water is run to waste for about 10 seconds in order to displace the water in the tap. The tap is disinfected as described in sections 6.3 or 6.4 and a sample taken as described in section 6.5.

6.9 Dip sampling

At times, dip sampling may be necessary for taking samples for investigational purposes. These samples should, however, only be taken where no alternative or more appropriate procedures are available. Dip sampling should not be used for regulatory drinking water compliance samples.

A dip sample may be taken for microbiological analysis using a sterile dip bottle. Sterile dip apparatus and sample bottles can be prepared using wide mouthed 500 ml sample bottles, normally attached to a wire or chain of sufficient length. The wire or chain can be attached either directly or via a bottle cage. Alternatively, clean sterile rope or string can be attached to the sample bottle or cage, but care should be taken to ensure they do not become contaminated or harbour microbiological growth. 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. The outer wrapping is removed from the bottle and then dipped into the water until filled. The lid is then carefully placed in the neck of the bottle.

Commercially available sterilised single-use dip samplers that are mounted onto short rod handles may be suitable for taking dip samples from small tanks or similar sample points.

7 Sampling from public buildings

The provision whereby a Member State is deemed to be in compliance with the European Directive⁽⁸⁾ when the cause of a failure to meet the standard at a tap, normally used for the supply of water for human consumption, is a deterioration in water quality within consumer premises, does not extend to premises, such as restaurants, schools and hospitals, where water is supplied to the public. How the requirements, to meet the standards at taps normally used for the supply of water for human consumption in such premises, will be transposed into UK legislation has not yet been finalised. However, it may be that regulatory drinking water compliance sampling in premises that supply water to the public for human consumption will include a requirement that the sampling tap should not be disinfected before a microbiological sample is taken. This will enable the effect of any storage and internal plumbing on the quality of water supplied at the tap to be assessed.

8 Sampling from aircraft, ships and trains etc

A suitable tap should normally be available for sampling in such places as aircraft, ships and trains etc, however, it is equally important to understand the supply arrangements to the tap that is used for sampling. The procedure described in sections 6.3 and 6.5 should be followed. For safety reasons, flame disinfection is not advised at these locations. Occasionally dip samples may need to be taken from storage tanks and the procedure described in section 6.9 should be used.

9 Sampling from private water supplies

The sampling procedures described in section 6 are equally applicable to both public and private water supplies. Before sampling a private water supply, it is important to be aware of details of the water source, any treatment and distribution arrangements etc in order to ensure that the water sampled is representative of the private water.

10 Drinks-vending machines

Most drinks-vending machines are often permanently connected to a water supply system. Some, however, may be hand-filled. Most plumbed-in machines do not possess facilities for flushing water to waste, and when these machines are used at the start of each day they are likely to draw water that has been standing overnight in the supply pipe-work. It may, therefore, be inappropriate to sample the water entering the machine after running water to waste.

Sampling arrangements will vary according to the design of the machine. If possible, samples should be taken from the supply pipe entering the machine without running any water to waste from the cold water storage tank, if fitted, and from the vended water from the cup station. For hand-filled machines, samples should be taken from the cold water storage tank and of the vended water from the cup station.

Further details and additional guidance are given elsewhere⁽⁹⁾.

11 Transport and storage of samples

Once taken, microbiological samples should be transferred immediately to dark storage conditions and kept at temperatures between 2 - 8 °C for transport to the laboratory. Insulated cool boxes or small refrigerators or refrigerated vans are suitable. Insulated cool boxes and small refrigerators used for the transport of microbiological samples should be used exclusively for this purpose. The inside of insulated cool boxes and small refrigerators should be kept clean and dry, and should be regularly disinfected. Van storage racks or boxes should be treated similarly. If samples are not analysed immediately on receipt in the laboratory, they should be kept at temperatures between 2 - 8 °C, in dark conditions until analysis commences.

Samples should be analysed as soon as practicable on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

There have been a number of studies^(10, 11) to assess the effects of transport and storage on microbial levels in water samples. Although refrigeration is important, the effects of storage time between sampling and commencement of analysis are influenced by many factors including the numbers and types of organisms present, the viability of organisms affected by disinfectants and water chemistry. Clearly, the longer the delay between sampling and commencement of analysis, the more likely that change will occur within the sample.

12 References

1. The effect of sodium thiosulphate on the coliform and *Bacterium coli* counts of non-chlorinated water samples. Public Health Laboratory Service Water Subcommittee. *Journal of Hygiene*, 1953 **51**, 572-577.
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3. Standing Committee of Analyst, Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts in waters 1999, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.
4. The Water Supply (Water Quality) (Amendment) Regulations 1999. Statutory Instrument 1999 No. 1524.
5. Standing Committee of Analyst, Methods for the isolation and identification of human enteric viruses from waters and associated materials 1995, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.
6. The Water Supply (Water Quality) Regulations 2000. Statutory Instrument 2000 No. 3184. Associated protocols are available from the Drinking Water Inspectorate at Ashdown House, 123 Victoria Street, London, SW1E 6DE or www.dwi.detr.gov.uk/regs/crypto/index.htm.

7. The Water Supply (Water Quality) Regulations 1989. Statutory Instrument 1989 No. 1147.
8. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Official Journal of the European Communities*, 5.12.98, L330/32-L330/53.
9. AVA (2000) Industry Guide to Good Hygiene Practice: Vending and Dispensing Guide Supplement (To the Catering Guide). Automatic Vending Association, London, Chadwick House Group Ltd.
10. Effects of transit time on indicator organism counts from water samples. *Microbiology Digest*, Tillett, H. E. & Benton, C., 1993, **10**, 116-117.
11. Effects of storage on analysis results for the total and faecal coliform parameters. 1994, DWI0314. WRc report to the Department of the Environment. London, Drinking Water Inspectorate.

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

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