



COLLECTION AND TRANSPORTATION

INSTRUCTIONS FOR MICROBIOLOGICAL WATER SAMPLING

1. SUPPLY OF SAMPLING EQUIPMENT

All water samples should be collected in bottles supplied by PathWest. Samples bottles/jars must contain sodium thiosulfate to neutralize any oxidant residual present in the sample (eg. chlorine, chloramine, bromine or ozone). This also applies for ice samples prepared from disinfected water. If you are unsure of the presence of a disinfectant, sample into a bottle/jar containing sodium thiosulfate. Where commercially pre-packaged water and similar products require analysis, the samples should be submitted in their original containers.

Sample bottles, foam coolers and ice-bricks can be collected in person from the laboratory at no charge. Alternatively, they can be obtained by completing the Supply Order form - see [MWF024](#). The cost of transporting the supplies is the responsibility of the client.

2. DOCUMENTATION

Water Examination Laboratory Request Forms must be submitted along with water samples. The laboratory prefers that these forms be used as they contain specific information that is required on reports. These Request Forms can be obtained from the PathWest website <http://pathwest.health.wa.gov.au/CommercialClients/Food%20and%20water/Pages/default.aspx>. The request form should be labelled with:

- Sender's name, address and contact details-Example: phone and email address (Shire, City council / Company etc.)
- Date sampled (date samples were collected)
- Order No. (sender's purchase order number if required)
- Sampled by (name of person who collected the samples, if required)
- Sample details - description of sample point and source of water
- Time of collection

Any extra information relating to the samples can be written in "Sender's Comments", i.e.: anticipated test ranges, dilutions required and test methodology (if known). Any

extra information relating to specific analysis required can be written in “Please specify test required if not routine”.

The sample bottle should be labeled with:

- Date sampled (date samples were collected)
- Sample details - description of sample point and source of water (written exactly as per the sample details provided on the request form)
- Time of collection

3. CHOICE OF SAMPLING SITES

The objective in collecting samples is to obtain a small but representative portion of the body of water being investigated.

The sampler must be aware of all the potential sources of pollution that may occur to a particular body of water.

4. ORDER OF TAKING SAMPLES

When a number of samples for various purposes are being collected from the same sampling point, the samples for microbiological examination should be collected first. Composite samples are not suitable for microbiological analysis.

If analysing for free-living amoebae as well as bacteria, two water samples must be collected.

5. OPENING AND FILLING THE BOTTLE

It is important to wash hands prior to sampling. Ensure that the sampler’s hands do not come in contact with the inside of the cap or the neck of the bottle. Sterile gloves may also be worn. Protect the sample from splashing and air drifts. Label the bottle before the sample is taken. A small air space of approximately 1-2cm needs to be left when the sample is collected to enable thorough mixing in the laboratory. The bottle must not be opened until immediately before sampling. To take the sample, hold the base of the bottle with one hand, remove the lid with the other hand and take the sample. Immediately replace the lid and secure it. The lid must not be placed on the ground. If accidental contamination is suspected, discard the sample and collect another sample in a fresh bottle. Do not use this sample for the measurement of temperature or any other on-site tested parameter.

6. SAMPLING WATER IN THE DISTRIBUTION MAIN

If the sample is required as part of an investigation into a consumer complaint, a sample should be taken prior to disinfection as well as following disinfection.

Prior to sampling, remove all external fittings such as hoses, clamps, screens and splashguards. Clean the outside and inside of the tap ensuring that any grease, dirt etc is removed and fully open and close the tap repeatedly to rinse out the dirt from the tap. Run water to allow sufficient time to flush out any stagnant water in the pipe. Turn off the tap and disinfect it by one of the following methods.

1. Disinfection by flaming PREFERRED METHOD

Disinfection by flaming is suitable for metal taps. The tap should be flamed thoroughly using a butane or propane burner. Methylated spirit flames should not be used. Start flaming at the nozzle and work back towards the body of the tap until the water held in the nozzle boils.

Subsequently, open the tap to half-flow and flush until constant water temperature is reached. Then place the open bottle in the water flow and fill it under aseptic conditions. Take care to avoid splashing or allowing water into the bottle which has made contact with the hand.

NOTE: Aseptic technique means using practices and procedures to prevent contamination from bacteria. It involves applying the strictest rules to minimize the risk of infection or contamination.

2. If flaming is not possible :Disinfection with 1g/L hypochlorite solution or 70% ethanol or 70% isopropanol.

NOTE: Health and Safety considerations must be adhered to when using chemicals.

Disinfection using a 1g/L hypochlorite solution or 70% ethanol or 70% isopropanol is suitable for plastic taps, mixer taps and metal taps where flaming is not appropriate. The outside of the tap, and as much of the inside as possible, should be dipped into, swabbed or sprayed with the solution and then left for 2 to 3 minutes. Subsequently, allow the water to flow long enough to minimize the influence of the network inside the building. It is necessary to know the detailed layout of the network (volume of tanks or softeners and retention time) to determine the flush time before sampling. Water temperature stabilization may be monitored to achieve the same effect. To minimize the effects of microorganisms that may have possibly been dislodged in the biofilm and debris, open the tap at maximum flow for 5-10 seconds, then reduce to half flow for 5-10 seconds, then place the bottle under the tap without closing and re-opening the tap, and take the sample. Take care to avoid splashing or allowing water into the bottle which has made contact with the hand.

Leaking taps should not be sampled for microbiological analysis until the leak has been fixed.

7. SAMPLING WATER AS IT IS DELIVERED TO THE CONSUMERS TAP

To determine the quality as it is delivered to the consumers' tap, ensure that no contamination from the outer surface of the tap reaches the sample. Clean the outside and inside of the tap ensuring that any dirt (scale, slime, grease or any other extraneous matter) which could fall off is removed before filling the bottles. Do not sample taps with leaking spindles. Take out any faucet nozzle or other attachment. Disinfect the tap preferentially by flaming or, if not possible, by other adequate methods (see Section 6 above). Subsequently, allow the water to flow just long enough to ensure the sample has no residual thermal or disinfectant effect. Place the bottle under the tap without closing and re-opening the tap.

8. SAMPLING WATER AS IT IS CONSUMED

To determine the quality of the water as it is consumed (e.g. in outbreak situations), contamination of the water by bacteria from the outside of the tap and from any attachment or device should be taken into consideration. Therefore, attachments should be kept in place and the tap **should not** be disinfected prior to sampling.

9. SAMPLING FROM WELLS AND SPRINGS (REFER TO AS 2031 FOR MORE DETAILED INFORMATION)

If the sample is to be taken from a ground source that is fitted with a pump, the pump should be continuously operated for at least 5 minutes before the sample is taken. The mouth of the pump is then heated, preferably by means of a blow lamp or gas torch, then cooled by pumping water to waste for a few seconds. The sample should be taken by allowing the water from the pump to flow directly into the bottle. If the sample is from a well or bore from which pumping is mechanical, the sample should be collected from a tap, previously sterilised, on the rising main, or from a nearby tap before the water enters a reservoir or tank.

10. SAMPLING OF ICE

Due to the fact that water expands when frozen, a larger volume of ice needs to be collected to supply to the laboratory for testing. The volume will depend on the tests required. In general if *Escherichia coli* only is required collect ONE (1) full sterile x 1 kilogram jar containing sodium thiosulfate. This is the testing usually required on ice for hospitals and hospital catering.

If the sample is from a commercial ice producer, then collect THREE (3) sterile x 1 kilogram jars containing sodium thiosulfate, full of ice to obtain 1000mL of melted water required for

testing. This testing often includes *E. coli*, *Pseudomonas aeruginosa*, total coliforms and a plate count. Contact the laboratory if you require guidance.

Transfer the ice into the container either by using the container as a scoop or by using a flame-sterilised spoon or collecting directly into the jar if it is an on-demand ice machine. Avoid touching the ice with hands. Sterile gloves may be worn.

11. COLLECTION OF SOIL, SEDIMENT, SLUDGE AND DUST

These samples can be collected in wide mouth sterile plastic containers that are available from the laboratory. The transfer of material into these containers can be done directly by using them as scoops or by using a flame sterilised spoon. Approximately 50g of sample is usually required for analysis. Contact the laboratory if you wish to submit samples for these matrices.

12. SURFACE WATER (E.G. NATURAL WATER, DRAINS, RESERVOIRS, TANKS AND SHALLOW WELLS)

- Move to the sample location and wade knee deep into the water, trying to avoid stirring up bottom sediment.
- Hold the sterile bottle in one hand near the base, and carefully remove and hold the screw cap with the other hand. Be careful not to touch the inside of the screw-cap when sampling.

OR

- Place the sampling bottle onto the sampling pole, making sure it is securely clamped into position. Carefully remove and hold the screw cap with your free hand. Extend the pole out into the water.
- Plunge the sample bottle neck downwards approximately 30 cm below the water surface, moving the bottle away from your body. This sample depth should be adhered to consistently.
- Turn the bottle neck slightly upwards to allow air to exit, which enables the bottle to fill up. Move the bottle into the current, remembering to keep your hand away from the mouth of the bottle at all times. The current should fill the bottle to the rim. If there is no current, move the bottle horizontally through the water until it is almost full.
- Once the bottle is full remove it from the water. A small air space of approximately 1-2cm needs to be left when the sample is collected to enable thorough mixing in the laboratory.
- Carefully replace the screw-cap immediately and tightly.

- **Note:** Use a sampling pole if it is not practical or safe to wade into the water (e.g. if the water conditions are turbulent, or if you are unsure of the physical hazards in the water.)

13. RECYCLED WATER

There are potential health hazards associated with the use of recycled water. These arise from the pathogens that may be present in raw sewage which are removed to varying degrees during treatment processes.

- Turn the tap on full flow and allow the water to run to waste for 2 minutes. This flushes the interior of the nozzle and discharges stagnant water from the pipe.
- Turn off the tap. Disinfection by flaming is suitable for metal taps. The tap should be flamed thoroughly using a butane or propane burner. Methylated spirit flames should not be used. Start flaming at the nozzle and work back towards the body of the tap until the water held in the nozzle boils. Allow the tap to cool by running water to waste for a few seconds and then take the sample ensuring that the bottle is filled with a gentle stream of water. Subsequently, open the tap to half-flow and flush until constant water temperature is reached. Then place the open bottle in the water flow and fill it under aseptic conditions. Take care to avoid splashing or allowing water into the bottle which has made contact with the hand.
- A small air space of approximately 1-2cm needs to be left when the sample is collected to enable thorough mixing in the laboratory.
- Carefully replace the cap.

14. SWIMMING POOL WATER

Telephone the WA Department of Health, Aquatic Amenities Unit on (08) 9388 4819 for instructions.

15. TRANSPORTATION OF SAMPLES TO THE LABORATORY

The time elapsing between the collection of samples and processing within the laboratory (known as the holding time) should not exceed 24 hours for most microbiological testing. If samples cannot be transported to the laboratory within one hour, they should be maintained at an appropriate temperature. This helps minimize the changes that may occur in the water sample during holding time.

Samples requiring bacteriological analysis must be sent in a cooler containing an adequate number of ice-bricks, aiming for a transport temperature of less than 8°C.

The samples must not be frozen.

For samples requiring amoeba analysis, the samples must be sent in a cooler (NO ICE BRICKS) at AMBIENT (room) temperature. The cooler must not contain any ice-bricks. Recommended and acceptable holding times and temperatures for organisms are listed in AS2031-please refer to this table. Samples should be dispatched to arrive at the laboratory between 8am and 3pm on Monday to Friday. Samples arriving outside of these hours on Monday to Thursday may be processed the following day and samples arriving after 3pm on Friday or on the weekend/public holidays will only be processed if prior arrangements have been made with the laboratory. Please contact the Senior Scientist on (08) 6457 2583 or (08) 6457 4372 to arrange for receipt and processing of urgent samples, or samples requiring processing outside of normal business hours (i.e. weekends, public holiday).

Samples sent to the laboratory should be addressed as follows: Waters Examination Laboratory, Environmental Microbiology Unit, Second Floor, J Block, Hospital Ave, Nedlands, WA 6009.

16. REFERENCE

Australian Standard AS2031- Water quality-Sampling for microbiological analysis.

17. DOCUMENT INFORMATION

Two printed copies of this document exist in the laboratory manuals. This document is also available electronically on Pathlines.

18. DOCUMENT SUMMARY

December 2017- Updated procedure to follow and reference AS2031

Significant changes made since last version:

September 2021 - Updated ice volume requirements.