

3.3.10 Collecting Samples for Microbiological Analysis

Purpose

The purpose of collecting samples for microbiological analysis is to assure that the water dispensed to the public is microbiologically safe to drink and to assure that samples submitted by the water system represents the water dispensed to the public.

Forms

- Sample chain of custody card for public water systems included in the sample container

Equipment

- Butane torch and torch lighter
- Squeeze bottle filled with a chlorine bleach solution
- Plastic bucket and containers to catch bleach runoff and flushing water
- Paper towels
- Sample container and mailing box provided by the Missouri Department of Health
- Portable chlorine test kit
- Tools to remove faucet aerators

Introduction

The Missouri Safe Drinking Water Law 640.115(5) authorizes representatives of the department to enter public or private property to inspect and investigate conditions relating to the construction, maintenance and operation of a public water system and to take samples for analysis. This provides the authority for department representatives to collect samples for a variety of tests. In addition, the Missouri public expects the department to collect samples and perform tests to validate that water samples taken and tests done by the water systems represent the water they dispense to the public. Samples should be collected whenever a department representative is conducting an investigation (unsafe sample, complaint) or inspection of a water system and their collection is especially important with water systems that do not disinfect. Samples may be collected anywhere in the water system where a suitable sample tap can be found. It is not necessary or at times appropriate for department representatives to collect samples at the routine sample locations specified in the system bacteriological sampling plan.

Some people believe that any samples collected by department representative should be marked special so that they will not be counted toward compliance. The regulations 10 CSR 60-4.020 (1) do allow for special purpose samples, such as those taken to determine whether disinfection practices are sufficient following pipe placement, replacement or repair and state that these sample results shall not be used to determine compliance. However, marking a sample “special” that was collected to determine if the water is ordinarily microbiologically safe to drink is not consistent with the intent of the regulation. Furthermore, the Missouri Public Drinking Water Regulation 10 CSR 60-4.010(5) requires that the results of all analyses be used to determine compliance with the maximum contaminant level, unless the analytical results are invalidated for technical reasons, such as obvious sampling errors. Thus, most samples collected by department representatives should be marked as routine samples and used to determine compliance. This places a heavy responsibility on the department representatives collecting samples. It requires us

to be painstakingly careful to follow correct sampling techniques and protocol. It requires us to make certain that the sample we collect accurately reflects the quality of the water in the distribution system. Furthermore, department staff should be an example to system operators of the correct way to collect samples for microbiological analysis. There are a few times when samples collected by department representatives should be marked “special” such as after shock treating a well or emergency disinfecting a distribution system to see if additional treatment is necessary. Some department staff mark samples as “special” if they are uncertain if the sample tap cannot be adequately sterilized. Since these samples would be considered invalid, it is better not to collect from these taps.

Tap Selection

The first step in collecting samples for microbiological analysis is to select the sample tap. Samples should be taken from a clean, smooth-nosed, cold water tap, free of attachments (hoses, aerators, screens, etc.) as shown below. Unfortunately, smooth-nosed, cold-water taps are



becoming difficult to find. Often, the best alternative is a standard hose bib or ball valve (as shown below). Some combination hot and cold faucets can be used to collect samples if the maximum temperature of the faucet is not controlled. (Faucets where the maximum temperature is limited to 115°F or less are ideal growing media for Legionella and other temperature tolerant bacteria and should not be used.) Any of these can be used to collect a water sample with appropriate precautions.



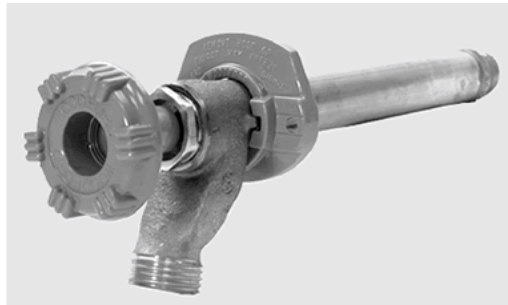


Some water taps should be avoided and not used for sample collection. Dripping faucets or those that leak around the valve stem or faucet base should be avoided. These are difficult to disinfect and have obvious avenues of contamination. Swinging faucets and faucets with risers (shown left) should be avoided because the mechanism that allows them to swing can also allow contamination into the water. Also, water constantly stands between the valves and the discharge of these faucets. This standing water can grow bacteria and other organisms.



Another water tap that should be avoided is a yard hydrant of any type (shown left). To keep them from freezing yard hydrants have weep holes or other mechanisms to drain the water standing in the risers. These drainage mechanisms allow surface water to enter the hydrant riser contaminate the hydrant and grow bacteria and other organisms.

Another type of water tap that should be avoided is a freeze proof sillcock (shown below). These are often called hose bibs but are significantly different from a standard hose bib. To keep from freezing the valve that controls the faucet is located inside of the house 16 to 20 or more inches away from the faucet end of the sillcock. Freeze proof sillcocks should be installed at an angle to allow water to drain from the



sillcock. However, many are not installed properly and do not drain well enough to prevent bacteria and other organisms from growing in the sillcock barrel. If a hose is attached to the sillcock it can stop the sillcock from draining and water from the hose can back up into the hose bib and contaminate the sillcock. Moreover, just the process of attaching a hose to the hose bib can contaminate the hose bib.

Do not collect samples from outside faucets that are closely surrounded by tall vegetation since the opportunity for contamination during sampling is very high. Do not collect samples from portable devices installed in meter wells. It is very difficult not to contaminate these devices during transportation and installation and disinfecting and flushing the device is also difficult. If the sample tap is outside and it is raining or the wind is blowing hard do not collect the sample. It is very difficult to keep from contaminating the sample container under these conditions. All sampling stations are not created equal and some are no better than yard hydrants. Thus, even sampling stations must be viewed critically before collecting a sample.

Before deciding to collect a sample at a customer find out if they have a softener or other type of point of entry or point of use treatment unit. Contaminated salt due to unsanitary handling or storage can cause positive samples after softening units and some other treatment units can cause positive samples. In systems that disinfect, the treatment units can result in no chlorine residuals in the customer plumbing. Try to find a tap before the treatment unit or do not collect a sample at this location.

Procedures Prior to Sample Collection

There is a debate as to whether sample taps should be sterilized or disinfected prior to collecting samples. Some quote Standard Methods as not requiring tap sterilization. However, Standard Methods does recommend flame sterilizing or disinfecting the sample tap if cleanliness of the tap is questionable. Since the department does not have control of any of the sample taps and cannot vouch for the cleanliness or any tap, it is the policy that all sample taps will be either flame sterilized or disinfected prior to sample collection.

Preparation for Collecting a Sample

- 1. Assemble all of the sampling supplies before you begin.** The containers are sterilized, so handle them carefully. Wash your hands thoroughly before handling supplies.
- 2. Because they may harbor bacteria and interfere with tap disinfection, remove aerators, strainers, or hoses that are present on the sampling tap.** If you use pliers or other tools to remove an aerator or strainer, be very careful not to mar or damage it. Wrapping the device in a paper towel helps but if the device is not easily removed choose a different sample tap.
- 3. Open the cold water tap for about 3 minutes before collecting the sample-** enough time to clear the service line. (You may want to time this step—3 minutes is a long time.) If the system disinfects continue to run the tap until the maximum chlorine residual is obtained and write the result on the sample card. If you are using a combination hot and cold water faucet, first run the faucet on hot until the maximum water temperature is obtained and continue to run hot water for an additional minute. (The maximum hot water temperature must be greater than 115 °F or choose a different tap.) Then follow the above procedure with the cold water as if it is a cold water faucet. This procedure requires the flushing of a lot of water, which can create a major mess if a drain is not available. Without a drain, the water must be run into a bucket and disposed of safely.
- 4. Flame-sterilize the tap from its nose to the valve.** Do not flame-sterilize if tap is plastic or if aerators are attached or if it causes a fire hazard. Be careful not to damage the faucet washers or valve sets. Short nosed taps are so vulnerable to damage that flame sterilization may not be appropriate. The finishes (chromium, antique, colored) on many faucets will not withstand flame sterilization. So, if you have any doubts do not flame-sterilize. If you cannot flame sterilize the tap, you may disinfect the tap by thoroughly rinsing both the inside and outside with at least a 100 ppm sodium hypochlorite (NaOCl) solution - mix ¼ ounce (1.5 teaspoon) of household bleach with one gallon of sterile water. A squeeze bottle filled with a bleach solution is the easiest way to apply the solution and to get the solution inside the faucet to the valve. Strong bleach solutions can damage things so try to keep the bleach contained to the sample tap and protect things around the tap. If the tap is dirty, wipe it off

with paper towels and let the bleach solution remain in contact with tap for up to 15 minutes. If you increase the strength of the chlorine solution, a shorter contact time with the chlorine solution can be used.

5. **In ink, fill out water analyses card completely and legibly.** The correct date and time is critical and be sure to use military time.
6. **Flush the tap for an additional 2 to 3 minutes and reduce to a gentle flow.** Check for steady flow, about the width of a pencil. Do not change the water flow once you have started sampling. It could dislodge microbial growth.

Sample Collection Procedures

The following steps describe sample collection procedures for coliform analysis and are only to be performed if Steps 1-6 have been completed.

7. **Grasp cap along top edge and remove carefully.** Be sure not to touch the inside of the cap or the bottle lip with anything. Hold the bottle in one hand and the cap in the other. Do not lay the cap down. Do not hold the cap upside down where dirt or other contaminants can fall or blow into the cap. If you think you may have contaminated the bottle or lid during sampling empty the bottle and get another bottle. Do not put the lid on the contaminated bottle but put the bottle and lid in the sample box and return it to the laboratory for sterilization. Do not rinse out a bottle before collecting the sample! The liquid or crystals inside the bottle are dechlorinating chemicals and are necessary for collecting valid samples.
8. **Allow gentle flowing cold water (pencil width) to fill bottle to the shoulder of the bottle.** Hold the bottle so that water will directly enter the bottle will not come in contact with anything else first. Samples will not be tested if there is less than ½ inch air space in the bottle, so do NOT overfill.
9. **Place the cap on the bottle and screw it down tightly.**
10. **Turn the tap off.** Replace any aerator, strainer or hose.
11. **Complete the bacteriological water analysis card** with the necessary sample collection information. **Is certain time and date are correct.** Fill out a separate water analyses card for each water sample submitted. Supply all information requested on the form and enclose with the sample bottle. Be certain that the number on the form matches the number on the bottle. **Samples with incomplete collection information could be invalidated and not tested.**
12. **Samples must reach the laboratory within 30 hours of collection,** from sample collection till initiation of analyses.

Shipping Samples

Samples require 18-24 hours of incubation before results can be determined and the results must be determined after no more than 30 hours of incubation. This limits when a sample can be

collected because the Department of Health Laboratories will not set up samples received on the weekends. Thus, samples must be received at the State Public Health Laboratories or the Springfield-Greene County Lab by 2:00PM Friday or they will not be set up. If samples are shipped by mail, they may be collected Monday through Thursday but not Friday. Collect samples on Friday only if they are hand-delivered to the Laboratory by 2:00 PM that day. Samples should not be in route to the laboratory over a weekend or state holiday. So, adjust your sampling to accommodate holidays.

Ship samples immediately after collection. This is important because the holding time for coliform samples is 30 hours. Check with your local postmaster for time of dispatch and collect the samples shortly before shipment to the laboratory. To ensure shortest shipping time, **use priority postage.** In some areas of the state, the US Postal Service has not been able to deliver samples within the 30-hour limit. In these areas you must either take the samples to another area for mailing, use the Department of Health and Senior Services contract courier or hand deliver the samples to the laboratory.

If using the Department of Health and Senior Services contract courier to deliver your samples, collect samples Monday-Thursday and have them dropped off at the courier pick up point the same day of collection, by 10:30AM unless a special arrangement is made. The contract with the courier specifies that the courier pickup cannot be made before 10:30AM. If collected and dropped off before 10:30AM, samples should meet the 30 hour holding time.

In special circumstances, samples may be sent by the courier service, on Fridays. Contact the appropriate Department of Health and Senior Services Laboratory to ensure a lab analyst is informed of the samples to be delivered and is available to set the samples up.