

# WATER SAMPLING

## WATERBORNE PATHOGENS

Water systems must strive to keep their drinking water free of disease causing organisms known as pathogens. There are five waterborne pathogens that can be found in all water supplies and two that are only found in surface water supplies. The protozoa that are found only in surface water supplies (*Giardia* and *Cryptosporidium*) form cysts and spores that protect them from cold temperatures. Because of this protection, they are much more difficult to kill with disinfectant chemicals.

### DISEASES CAUSED BY WATERBORNE PATHOGENS

#### All Water Sources:

Typhoid  
Paratyphoid (Types A & B)  
Cholera  
Dysentery  
Hepatitis

#### Surface Water Only:

Cryptosporidium  
Giardia

Most of these diseases are caused by pathogenic bacteria. The exceptions are Hepatitis, *Giardia* and *Cryptosporidium*. Hepatitis is a virus. *Giardia* and *Cryptosporidium* are both protozoa. It is very difficult to identify any one particular pathogen by laboratory testing. To make testing more reliable and economical, the lab tests are designed to identify a large family of bacteria that are related to the disease causing bacteria, rather than identifying each type of pathogen.

## COLIFORM GROUP OF BACTERIA

Coliform bacteria are enteric bacteria. This means that they are found in the intestinal tract of warm-blooded animals, including humans. These bacteria do not cause disease but are necessary for the digestion of food. The waterborne pathogens are also enteric organisms. Some of the bacterial pathogens are part of the coliform family.

If coliform bacteria are present in the water supply, pathogens may also be present. The coliform bacteria live longer in water and are easier to detect by laboratory testing. This is the reason the coliform group has been chosen as the indicator organism for waterborne pathogens. If coliform bacteria are not present it is assumed there are no pathogens present either.

The coliform family has been divided into two groups. Results may come back as either total coliform positive (TC positive) or fecal coliform positive, or (FC positive or *E. coli* positive.) Total coliform positive means that no human coliform are present. Fecal coliform positive indicates the presence of *E. coli*, which means there is a greater chance of pathogens being present. The laboratory tests for coliform include the MPN method, the Membrane Filter test, the Colilert test, and the presence-absence test. Most of the certified labs in New Mexico use either the colilert or membrane filter (MF) test. These tests require  $100 \pm 2.5$  milliliters of sample.

## **MONTHLY SAMPLING**

It is the responsibility of the system to collect samples for microbiological ("Bac-T") testing. It is very important to make sure samples are collected and tested and results reported properly. If a sample becomes contaminated due to poor sampling procedures or is not sent to the testing laboratory at the proper time, the system may technically be in violation of the drinking water regulations. This may result in the system having to notify the public of violations when the water is actually safe.

Most state's Drinking Water Regulations regarding microbiological sampling schedules set the minimum number of samples a water system must submit at one per month. As the size of the system increases so does the number of samples required each month (maximum of 480).

## **THE SAMPLE SITING PLAN**

The sampling points for routine compliance sampling should be identified by a sample siting plan. The sample siting plan is created to locate sites for samples from all major parts of the system to assure that the entire system is represented over several months. Small systems must have at least two sample sites in the system.

Sample siting plans must identify the sample locations by address or description, when street addresses are not available. A map of the different sites in the system is also a requirement. A drawing of the specific location of the sample tap should also be included if necessary. The location of the sample site, once properly identified in the sampling plan, can then be referenced by location or address on the sample request form. Sample siting plans must be reviewed and approved by the state primacy agency. Sampling schedules should be set up to collect compliance samples during the first half of the month. This will allow time for repeat sample collection and testing, if necessary, before the end of the calendar month.

## THE SAMPLE BOTTLES

There are three types of sample bottles that are used by the majority of certified microbiological testing labs. Sample bottles are provided by the lab and are sterilized prior to distribution. The three types of sample bottles are:

**Nalgene** - These are the only reusable bottles. Larger municipal and state labs use them. They are sterilized by the lab and returned to circulation after each use.

**IDEXX** – These bottles are clear plastic disposable containers that are supplied by the manufacturer with the colilert testing kits. Many labs that use the colilert method also issue the IDEX bottles.

**Snap Lid** – These bottles have a hinged cap that has a snap-on type seal and a hinged latch to secure it. A plastic “Key” is used to secure the latch once the sample is collected.



**NALGENE**



**IDEXX**

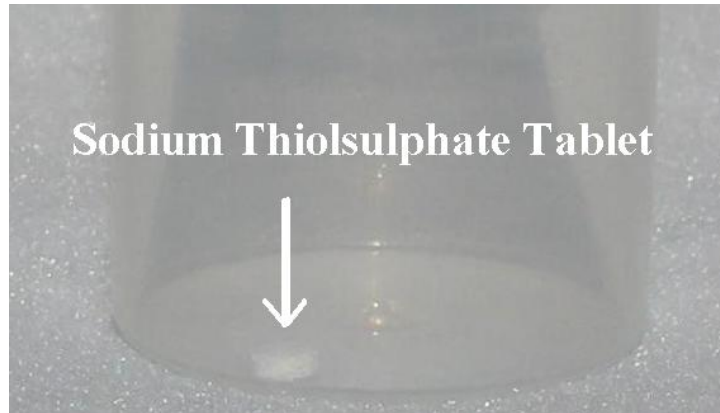


**SNAP LID**

## MICROBIOLOGICAL SAMPLE BOTTLES

## PREPARING TO COLLECT THE SAMPLE

The first consideration in collecting a "Bac-T" sample is the sample bottle. These bottles are provided by the laboratory. They are sterilized prior to being distributed. They should be stored in a cool, dry place until they are needed for a sample. Sodium thiosulphate is added to the bottle before it is sterilized. It may be in the form of a clear liquid, a white powder, or a white tablet in the bottom of the bottle. The purpose of the sodium thiosulphate is to neutralize any chlorine residual that is present in the sample.



Things to remember when preparing to collect microbiological samples:

1. Don't forget the chlorine residual test kit. If the system disinfects, a free chlorine residual needs to be present prior to sample collection. The residual must be recorded on the sample request form.
2. A cooler and blue ice packs (or regular ice) will be needed for sample preservation. If ice is used, plastic bags will be needed to keep the sample bottles and forms dry. Samples must be refrigerated to 4°C (39°F).
3. Use alcohol, soap, or latex gloves to prevent contamination due to dirty hands.
4. A pen should be used to fill out forms. Use tape and a permanent marker to label sample bottles.
5. Always take extra bottles and sample request forms. Repeat samples will also require red evidentiary seal tape.
6. Never wash out a bottle or even open it until you are ready to take the sample.
7. If a sample bottle has any dirt or junk in it or in the lid, don't use it. It's better to get a new bottle than to take a bad sample. See item 5.

## SAMPLE COLLECTION

The location of the sample should be determined by referring to the sampling plan. Once the site has been selected, the next consideration is the collection of a valid sample. The procedure for collecting the sample is given below. These instructions should be carefully followed to prevent accidental contamination of the sample.

1. **Select a sampling point.** Always keep sanitary conditions in mind when selecting a sampling point. Never use a kitchen sink faucet that swivels or an outdoor faucet that drips. Any hoses, vacuum breakers or other attachments must also be removed. The least-used faucet at the site is preferred because there is less chance of contamination of the faucet. If an indoor faucet is selected, make sure the sink and faucet are clean. Never collect a sample from a hot water faucet. Remove the aerator screen (it might be contaminated). If the sample tap is located in an open area, clean brush and other vegetation for 3-5 feet away from the sample site.

Disinfect the faucet with alcohol if necessary. Avoid using bleach to clean the faucet. It doesn't evaporate as quickly and spills are a bigger problem to clean up. And finally, wash your hands or put on latex gloves before collecting the sample.

2. **Flush the line.** Open the tap and let the water run for 3-5 minutes or until the temperature changes. This will insure that the water being sampled is from the main and has not been standing in the customer's plumbing.
3. **Take a chlorine residual reading.** Once the line is properly flushed, throttle the flow down to an unaerated stream. Run a chlorine residual analysis and record the free chlorine residual. It must be included on the sample request form.
4. **Collect the sample.** Remove the cap, making sure that you do not touch the inside of the cap or the top of the sample bottle. Don't aerate the sample or allow it to splash on the outside of the bottle. Don't touch the inside of the cap or bottle. Don't blow or breathe into the sample bottle. Hold the bottle at a 45 degree angle while filling it.

Fill the bottle carefully to the 'fill line' or within about 1" of the top. This guarantees that there is at least 100ml of sample in the bottle. Do not fill it all the way. Never pour excess water out of the bottle. If you overfill it, draw another sample with one of those extra bottles you brought. An air space is needed to agitate the sample before it is tested at the lab.

Never set the cap down or leave it off longer than it takes to collect the sample. Hold the cap so that it is facing down to avoid having debris settle in it. Replace the cap and make sure it is tight

5. **Mark the bottles for identification.** Mark the disposable bottles directly and use a piece of tape or other suitable label to mark and identify the reusable Nalgene bottles. The information on the label should include the address, date and time. If records are being data based, the computer reference number should also be included.
6. **Refrigerate the sample.** The sample must be refrigerated to lower the temperature to 39°F or 4°C until tested. Always place the sample and the form in a plastic bag if ice is being used to refrigerate the sample. This is a good practice even if blue ice is being used because condensation can occur. If the samples are wet, the lab may reject them because they can't be sure the sample didn't leak.

## **REPORTING AND SHIPPING CONSIDERATIONS**

The final consideration for microbiological sampling is the proper completion of the sample form and delivery to the lab. The following information must be included on the sample form:

### **Microbiological Sample Request Form Data**

- 1. System name, address, and identification number**
- 2. Location of sampling site**
- 3. Date and time sample was taken**
- 4. Type of water sampled**
  - a. Routine Sample – Compliance**
  - b. Repeat Sample**
  - c. Special sample – Line break/raw water**
- 5. Chlorine residual**
- 6. Reference number (if it's a repeat sample)**
- 7. Name of operator taking sample**

There are times when water systems need to collect microbiological samples for reasons other than monitoring compliance. New water lines and lines that have been repaired should be tested. Wells that have been disinfected should be tested. These samples must be identified as something other than a routine or compliance sample so they will not count as a violation against the system if they are found to be positive. This is done by checking “Special Sample” on the form.

There are certain requirements regarding the age of a microbiological sample. *Standard Methods* states that the sample must be tested within 30 hours after it is collected. If a sample does not arrive at the lab within this time period it will not be tested. Most labs require that the sample arrive at the lab within 24 hours of collection so the testing can be done before it is 30 hours old.

Some labs do not accept samples on Fridays, since they are not open on Saturday to read the results. This means that it is important to take compliance samples on a Monday. If positive results are received on Wednesday, the repeats can be run on Thursday. Remember that repeat samples must be taken within 24 hours of notification. If you receive notification of a positive sample on Friday and the repeat can't be run until the following Monday a violation has occurred.

## REPEAT SAMPLES

Anytime a microbiological sample result comes back positive, indicating the presence of total or fecal coliform/ *E.coli*, repeat samples must be taken. Three repeats are usually required. One must be taken at the site of the positive sample. The two samples must be taken upstream and downstream of the original site (within five service connections). These repeat samples must be taken within 24 hours of notification of positive results. They must be identified as a Repeat Sample on the sample form. Repeat samples may be required to be sealed with a red evidentiary seal tape. The tape must cover the cap and extend down the sides of the bottle. The sample forms must also include the reference number for the positive sample.



**Repeat sample  
with red seal tape**

There is an important exception to the three repeat samples rule. The regulations also state that when repeats are taken the minimum number of samples is raised to five for the month. **A system that collects just one sample a month must collect four repeat samples, when the sample is positive, in order to have five samples as required.**

**Whenever a system has to take repeat samples, a minimum of five routine samples must also be submitted the following month.** This is only an issue for systems that normally turn in four or fewer samples each month. If the five samples are negative the system can return to its normal sampling schedule the next month.

Small systems that have fewer than four sampling sites have a problem complying with the “upstream and downstream” aspects of the repeat sampling requirements. In this case, samples should be taken at as many separate sites as possible and then wait a minimum of 2 hours before resampling enough sites to get the required number of samples.

## VIOLATIONS

When repeat samples come back positive, the system is usually in violation of the microbiological standards (unless 40 or more samples are taken, then no more than 5% of the samples can be positive). If all of the positive samples were total coliform, it is a Tier 2 violation. If the MCL is exceeded and fecal coliform or *E. coli* positives occur in either the routine samples or the retakes, it becomes a Tier 1 violation requiring immediate public notification. In either case the primacy agency must be notified within 24 hours of any positive sample results. The water system is responsible for any public notification that may be required.

## INORGANIC SAMPLING

Analyses of inorganic contaminants are broken down into two different. Heavy metal contaminants like lead, copper, iron, manganese, and mercury, are included in one group. The other group includes analyses for non-metals (fluoride, chlorite/bromate, nitrate/nitrite, chloride, sulfate), hardness (calcium and magnesium), alkalinity (carbonates, bicarbonates) and other metals like (sodium and potassium.)

The samples for heavy metal analysis are collected in a 1 liter (1 quart) plastic cubitainers. Most of the other inorganic samples are either 1 liter or 100 ml samples. The 1 liter samples can be collected in the 1 liter cubitainers. The 100 ml bottles can be either plastic or glass. The Complete Secondary and Major Cation/Anion Groups are 4 liters.

Things to remember when preparing to collect inorganic samples:

- 1) A pen should be used to fill out forms. Use tape and a permanent marker to label sample bottles. Containers, forms, and ID labels are available from SLD.
- 2) A cooler and blue ice packs (or ice) will be needed for sample preservation. If ice is used, plastic bags will be needed to keep the sample bottles and forms dry.
- 3) Separate forms must be filled out for samples that going to each lab.
- 4) Remove screens and fittings from the faucet and flush the line for 3-5 minutes. **NOTE:** The exception this rule is the collection of Lead and Copper samples. They must be taken as a “first draw” samples after water has been standing in the plumbing for atleast 6 but not more than 18 hours.
- 5) Inorganic samples also require preservation by chemical adjustment of the pH. Most samples require a pH of 2.0 or less using nitric acid ( $\text{HNO}_3$ ) as the preservative chemical. Nitrite and nitrates samples are preserved using sulphuric acid ( $\text{H}_2\text{SO}_4$ ). Cyanide sample are preserved by raising the pH to 12 or higher using sodium hydroxide ( $\text{NaOH}$ ).

## ORGANIC SAMPLING

There are different sampling kits used to collect the various organic samples for testing. Many of the contaminants are related chemically and are grouped together for sampling and monitoring purposes. The chemicals are grouped into Volatile Organic Compounds (VOC), semi-volatile organic compounds (SVOC), and Disinfection By-Products (DBP).

Volatile organic compounds are those chemicals that evaporate quickly. The solvents like benzene, toluene, and carbon tetrachloride are included in this group. Others, like styrene and vinyl chloride, are used to manufacture petrochemical products and plastics. Semi-volatile organic compounds are the heavier chemicals that do not evaporate. Many of these chemicals, like lindane and 2,4-D, are used as pesticides and herbicides. Organic disinfection by-products sampling includes total trihalomethanes (TTHM) and haloacetic acids (HAA5). TTHMs are volatile organics and haloacetic acids are semi-volatile.

## PREPARATION FOR SAMPLE COLLECTION

Water is an excellent solvent for many organic compounds. When exposed to air, it has the ability to absorb volatile organic gases that may be present. There are special precautions that must be taken to avoid contamination of organic water samples. VOC I/II and THM samples are the easiest to contaminate. They must be collected with no headspace (air) in the bottle. Techniques used to correctly fill these sample bottles will be detailed with the individual collection instructions. Always observe the following precautions prior to collecting an organic water sample:

- 1) Check the sampling kit to make sure that all of the bottles and preservatives are present. Check the sample form(s). A marker or pen will be needed to fill out labels and forms.
- 2) Personal hygiene issues can increase the risk of sample contamination, especially when taking VOC samples.
  - A) No smoking – Tobacco smoke contains VOCs that can be absorbed by water.
  - B) No hairspray/mousse, cologne/perfume, or breath spray/mouthwash for the same reason as tobacco smoke.
  - C) Latex gloves should be worn during sample collection - Change gloves at each new sampling site.
- 3) Sample preservation will require refrigeration. A cooler with ice and a number of sealing plastic bags, to store sample bottles and forms, should be utilized.
- 4) Make sure all vehicle or other combustion engines are off and the area is well ventilated. VOCs in engine exhaust can also contaminate the samples.

- 5) Remove any aerator screens or other attachments from the faucet. Flush the faucet for 5-10 minutes to stabilize the water temperature.
- 6) Many of the samples require special preservative measures. The preservatives differ in the various sample. Hydrochloric acid is used in many of the VOC samples. Sulfuric acid, sodium thiosulfate, and citrate buffer may also be used in other cases.

The following procedure should be used to collection of VOC samples. (Repeat for both vials):

- 1) After the sample faucet has been properly flushed, reduce the flow to a trickle.
- 2) Remove the cap, hold the vial at a 45 degree angle. Position the vial as close to the faucet as possible and make sure the stream hits the side of the vial as it fills. Slowly fill the vial until it overflows the rim slightly.
- 3) Wait 20 seconds for any entrapped air to rise to the top. Then add 2 drops of 1:1 HCl using the dropper and acid provided by the lab.
- 4) Replace the cap without getting any air in the sample vial. There are two methods that can be used to do this:
  - A) Fill the vial until you get a convex meniscus (bulging over the rim). Carefully slide the septum across the top of the vial and then screw on the cap.
  - B) Fill the vial to get a convex meniscus and fill the cap (don't remove the septum) with water. Hold the inverted cap next to the rim of the vial and carefully flip the cap over the top and screw it down.
- 5) Check to make sure there are no bubbles in the sample. If bubbles are present, remove the cap, add a little more sample water and try again.
- 6) Record site data, date, and time on the site labels and place them on each vial. Place the lab-supplied ID labels to each vial. Place vials in plastic bag and seal. Refrigerate (4°C/39°F) during storage and transport. Samples must be analyzed within 14 days of collection.

## **RADIOLOGICAL SAMPLING**

There are few special sampling requirements for radiological sampling. Some samples may need to be preserved using nitric acid to achieve a pH of 2.0 or less. Samples are normally 1 or 2 gallons, depending on the sample protocol. Radon is the only sample that requires refrigeration at 4°C.

### **BASIC STUDY QUESTIONS**

1. Who is responsible for the sample siting plan?
2. Why is sodium thiosulphate used in a microbiological sample?
3. Which faucets should be avoided when selecting a sampling point?

### **BASIC SAMPLE TEST QUESTIONS**

1. Microbiological samples must be tested within \_\_\_\_ hours.  
A. 12  
B. 24  
C. 30  
D. 36
2. If a systems takes one microbiological sample a month, how many repeat samples must be taken when positive result are reported?  
A. 2  
B. 3  
C. 4  
D. 5
3. How many milliliters of sample are required for testing?  
A. 50  
B. 75  
C. 100  
D. 125

### **ADVANCED STUDY QUESTIONS**

1. Why should routine samples be collected early in the week?
2. Which samples require a red tape seal and chain of custody?
3. Which samples cannot have air bubbles in them?
4. Which chemicals for preserving inorganic samples?

### **ADVANCED SAMPLE TEST QUESTIONS**

1. If positive results occur, what is the minimum number of samples for next month?  
A. 1  
B. 3  
C. 5
2. Repeat samples require upstream and downstream sampling. This must be done within how many service connection of the original sample?  
A. 2  
B. 3  
C. 4  
D. 5
3. A sample collected after a water line repair should be identified as a \_\_\_\_ on the sample request form.  
A. Routine sample  
B. Repeat Sample  
C. Special sample  
D NMED monitoring sample

