Ohio Department of Health
Bureau of Environmental Health
Procedure for the Collection and Testing of Grab Samples as Required by NPDES General Permits for Discharging Household Sewage Treatment Systems

246 North High Street
Columbus, Ohio 43215

www.odh.ohio.gov
614/466-1390

Purpose

Statewide guidance concerning the collection and testing of effluent grab samples collected as part of the monitoring requirements in the Ohio Environmental Protection Agency (EPA) National Pollutant Discharge Elimination System (NPDES) General Permit OHK000001 or OHL000001 has been developed by the Ohio Department of Health with input from local health departments, system and component manufacturers, and the Ohio Sewage Treatment System Technical Advisory Committee. This guidance has been developed as a mechanism to ensure consistent, accurate, and safe sample collection across the state of Ohio.

In no way should the procedures outlined in this document be construed as contradictory or as a replacement to 40 CFR Part 136, *The Standard Methods for the Examination of Water and Wastewater,* or the US Environmental Protection Agency's *National Pollutant Discharge Elimination System Compliance Inspection Manual.* The mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use by the Ohio Department of Health.

Discharging sewage treatment systems operating under the coverage of the Ohio EPA NPDES general permits must be operated, and maintained in compliance with the requirements outlined within the permit. One monitoring requirement outlined in the general permits is the annual collection and testing of grab samples from all systems installed under their coverage. Effluent limitations and monitoring requirements are outlined in Tables A.1 and A.2 of the general permits and are provided in Appendix A of this document.

Sampling procedures and methods shall conform to 40 CFR Part 136.3, *The Standard Methods for the Examination of Water and Wastewater,* and the US Environmental Protection Agency's *National Pollutant Discharge Elimination System Compliance Inspection Manual* in all cases. Analysis of improperly collected or contaminated effluent samples will result in data that could lead to an incorrect conclusion regarding treatment systems operation and ultimately unnecessary homeowner expense.

Safety

Occupational Safety and Health Administration (OHSA) standards must be understood and followed when collecting wastewater samples. Eye protection, suitable and sterilized disposable gloves, and protective clothing must be worn and scrapes, cuts, and burns must be covered. Care should be taken to disinfect equipment, dispose of gloves properly, and wash hands thoroughly following sample collection. Always avoid eating, drinking, or smoking near samples, at the sampling location, and in the laboratory.

Qualified Laboratory

Choose a laboratory that is qualified to perform the testing required by the permit. A qualified laboratory is one that is familiar with and capable of following *The Standard Methods for the Examination of Water and Wastewater*. Contact the local health department, system distributor, local wastewater plants, and / or the sanitary engineer to locate a qualified laboratory. It should be noted that some laboratories will collect the grab samples themselves before performing the analyses. Ensure that the collection time and location will conform to the required holding times. Travel time to the laboratory, the laboratory's operating hours, and weekend or holiday schedules all need to be considered when collecting samples.

Methods for Grab Sample Analysis

Flow Rate (Daily)

When the system does not include flow measuring devices the flow rate should be calculated by multiplying the estimated daily flow rate of 60 gallons per day per capita by the number of residents living within the dwelling. When an accurate count of residents is not available, the flow rate should be calculated by multiplying the estimated daily flow rate of 120 gallons per day per bedroom by the number of bedrooms in the dwelling. An estimation of the systems daily flow rate should be accompanied with the calculations used to derive the estimation.

Total Suspended Solids (TSS), Carbaceous Biochemical Oxygen Demand ($CBOD_5$), Nitrogen, Ammonia (NH_3), fecal coliform, and E. coli

TSS, $CBOD_5$, NH_3, and bacterial concentrations are determined through the analysis of a correctly collected and preserved grab sample at a qualified laboratory. The appropriate laboratory procedures for testing for pollutants are outlined in 40 CFR Part 136.3 and *The Standard Methods for the Examination of Water and Wastewater*.

Dissolved Oxygen

The dissolved oxygen concentration may be determined by utilization of either the Winkler (Azide modification) or the electrometric method using membrane electrodes. When utilized, an electrode must be submerged into a sample collected directly from the effluent pipe in the inspection box within 15 minutes of the collection of the sample. Do not obtain a dissolved oxygen calculation from stagnant water inside the inspection

port. Discard sample effluent into the inspection box after attaining dissolved oxygen concentration.

Color, odor, and turbidity

Color, odor, and turbidity severity are determined and recorded by the individual performing the monitoring in the field using the following table from Part IV *Special Conditions* of the general permits.

TABLE I: Color, Odor, and Turbidity Severity

From OEPA General NPDES Permit OHK00001, OHL00001

For turbidity, odor, and color, use the following table to determine the value between 0 and 4 that is reported.

REPORTED VALUE*	SEVERITY DESCRIPTION	TURBIDITY	ODOR	COLOR
0	None	Clear	None	Colorless
1	Mild			
2	Moderate	Light Solids	Musty	Grey
3	Serious			
4	Extreme	Heavy Solids	Septic	Black

* interpolate between the descriptive phrases

Fats, oil, grease, or other floating material

The presence of sludge deposits, fats, oil, grease or other floating material must also be observed and recorded at the sampling site. Part V, I of the general permits states:

I. General Effluent Limitations. *The effluent shall, at all times, be free of substances:*

1. in amounts that will settle to form putrescent, or otherwise objectionable sludge deposits; or that will adversely affect aquatic life or water fowl;

2. of an oily, greasy, or surface-active nature, and of other floating debris, in amounts that will form noticeable accumulations of inappropriate scum, foam or sheen;

3. in amounts that will alter the natural color or odor of the receiving water to such degree as to create a nuisance;

4. in amounts that either singly or in combination with other substances are toxic to human, animal, or aquatic life;

5. in amounts that are conducive to the growth of aquatic weeds or algae to the extent that such growths

become inimical to more desirable forms of aquatic life, or create conditions that are unsightly, or constitute a nuisance in any other fashion; and

6. in amounts that will impair designated instream or downstream water uses.

7. that may result in public health nuisances, as defined by OAC 3745-1-04, associated with raw or poorly treated sewage.

Chlorine

Residual total chlorine may be determined by using a variety of methods including Methods 4500-Cl B-G *The Standard Methods for the Examination of Water and Wastewater*.

Temperature

Although an effluent temperature reading is not required by the NPDES general permits, identification of the temperature at the time of collection may be considered advantageous in some situations. When a temperature reading is identified it shall be determined by submersing a thermometer to its indicated immersion distance (typically a line etched around the stem of a partial-immersion thermometer) into a sample collected directly from the effluent pipe in the inspection box and taking a reading from the thermometer once it has reached equilibrium. A reading must be taken within 15 minutes of the collection of the sample. Do not obtain a temperature reading from stagnant water inside the inspection port. Discard sample effluent into the inspection box after attaining temperature reading.

Equipment

Ensure that you have access to the required instruments and that they are properly calibrated. Follow the *Quality Control* Procedures in *National Pollutant Discharge Elimination System Compliance Inspection Manual* Chapter 5 Table 5-4 and the manufacturer's instructions to ensure equipment is properly calibrated. All maintenance and calibration of testing instruments should be documented and submitted to the local health department with testing results.

Sampling Bottles

When collecting samples for bacteriological testing, it is important to use clean and sterilized glass or plastic sampling bottles. Use bottles with sufficient volume to collect the sample and provide an adequate air space. 40 CFR Part 136 outlines requirements for sampling bottle materials. Sampling bottles and caps should be obtained from the laboratory performing the analysis on the samples. It may be advantageous keep a supply of extra bottles of each size in case a bottle becomes contaminated during sample collection.

Dissolved Oxygen Meter

When the dissolved oxygen concentration is determined using the membrane electrode method, the meter must be capable of accuracy of ±0.1mg/L and a precision of ±0.05 mg/L. Follow the manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy.

Reagents

Ensure that all chemical reagents are prepared correctly and do not have an expired shelf life. Ensure that all reagents are stored correctly and safely.

Thermometer

A thermometer must have a scale marked for every .1C, with markings etched on the capillary glass and a minimal thermal capacity to permit rapid equilibration. Use a thermometer with a metal case to prevent breakage. Since the entire thermometer will not be submerged in most cases a partial-immersion thermometer is preferred.

Observations and Grab Sample Collection

Part IV B of the general permits states that *grab samples shall be collected at such times and locations, and in such fashion, as to be representative of the facility's performance.* Grab samples must be collected from a sampling port installed after final treatment of the effluent. Although other sample ports within the treatment train may be required by the manufacturer for maintenance purposes, all NPDES systems must have a sample port installed after final treatment. The local health department must be contacted if an appropriate sample port is not available.

A grab sample cannot be taken during periods of low or no flow. Contact the system manufacturer to determine the recommended procedure for inducing or capturing flow in these situations. If the manufacturer recommends inducing flow by turning on a faucet in the dwelling or by adding water prior to the first treatment component of the system, it must be representative of normal flow from the residence (typically no more than 5 gpm).

Record the color, odor, and turbidity severity using the table provided in this document from Part IV of the general permits. The presence of sludge deposits, fats, oil, grease or other floating material must also be observed and recorded at the sampling site.

The temperature, dissolved oxygen concentration, and chlorine concentration must be measured using the appropriate methods and recorded immediately, on-site.

The presence of chlorine will affect bacterial testing results. If chlorine is detected it must be removed or stabilized prior to storage and transport of the sample for laboratory analysis. Sample bottles containing Sodium thiosulfate, a dechlorinating agent, are available from the laboratory. The presence of a dechlorinating agent must be identified on the sample bottle.

All grab samples measurement should be collected over a period of time not to exceed 15 minutes. The sample volume depends on the type and number of analyses to be performed. 40 CFR Part 136.3 outlines requirements for sample sizes. Consult with the testing laboratory to determine the volume of effluent needed and appropriate bottle for each analysis.

Care must be taken when collecting the grab samples for laboratory analysis. The grab samples must be collected from a free falling stream at the end of the effluent pipe in the inspection port provided in all approved NPDES household sewage treatment systems. Do not collect the grab samples from stagnant water inside the inspection port.
Mark the sample bottles with the site address, time and date of collection, any additives introduced to the sample, and whether chlorine was detected before collecting the sample.

1. Do not remove the cap from the sample bottle until you are ready to take the sample.

2. Remove the cap from the sample bottle holding the exterior of the cap. Do not touch the rim or mouth of the bottle. Some sample bottles are sealed to ensure they remain sterilized. Be sure to remove the tape completely before collecting a sample. Contamination of samples to be analyzed for fecal coliform or E. coli will affect sample results.
3. Carefully fill the sample bottle.
    a. Sample bottles to be analyzed for fecal coliform or E. coli must be filled without rinsing. Leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Do not allow the sample bottle rim to touch the effluent piping or any other source of contamination.
    b. Overflow the airtight sample bottle to be analyzed for $CBOD_5$. The presence of oxygen in the airspace will affect these samples.
4. Recap the sample bottle. The bottle should only be open during the collection of the sample.
5. If there is any question whether the sample to be analyzed for fecal coliform or E. coli was contaminated during collection, discard the sample and start over at the first step.

Storage and Transport

The sample must be packed in ice and held at <4ºC for less than six hours for transport to the testing laboratory. Re-freezable ice packs are not sufficient to ensure compliance with the required holding temperature and are prohibited. Preferred holding times, from collection to analysis, are provided in the following table:

Table II— Collection and Preservation Techniques with Holding Times

derived from 40CFR (1998) Part 136, and Norweco, Inc.'s *Effluent Sampling For Residential Treatment Systems,* and *The Standard Methods for the Examination of Water and Wastewater*

Parameter Name	Preservation* (temperature, reagents)	Holding Time**	Sample Size***	Appropriate Air Space in Sample Bottle
Fecal Coliform or *E. coli*	Cool, <10°C 0.0008% Na2S2O3	6 hours	N/A	≥ 2.5 cm
Ammonia (NH_3)	Cool, ≤4 °C H2SO4 to pH<2	24 hours	500 mL	N/A
Carbonaceous biochemical oxygen demand ($CBOD_5$)	Cool, ≤4°C	6 hours	1,000 mL	0 cm - The sample must be void of airspace.
Total Suspended Solids (TSS)	Cool, ≤4ºC	24 hours	200mL	N/A

* Reagents may be needed in the presence of chlorine. Discuss reagent use with the qualified laboratory.
**Preferred holding time from collection to analysis. Consider laboratory hours and schedule when delivering samples. All samples should be analyzed as soon as possible after collection
***Check with the qualified laboratory for sample bottle size; sample size may be dependant on analysis methods

Chain of Custody

Written records must accurately trace the custody of each sample through all phases of the monitoring program. The primary objective of a chain of custody is to create an accurate written record that can be used to trace the possession and handling of the

sample from the moment of its collection through its analysis. A chain of custody form may be obtained from the laboratory performing the analysis on the samples or sample chain of custody forms can be found in the USEPA *National Pollutant Discharge Elimination System Compliance Inspection Manual* Chapter 5, Appendix M or in Appendix B of this document.

Reporting and Records Retention

The results of all analyses and measurements shall be submitted to the local health district having jurisdiction and shall be retained by the health district for inspection. The results of all analyses and measurements must be accompanied by the site address, homeowners name, time, and date of the sampling, the specific sampling location within the treatment train, the name of the person(s) and company collecting the samples and measurements, the name of the person(s) and laboratory who performed the analyses, and the analytical techniques or methods used. Maintenance and calibration schedules and records for all testing instruments must accompany the testing results.
Part V N *Records Retention* of the general permits states that local health district shall retain all of the following records for a minimum of three years (unless otherwise noted), including:

1. *all sampling and analytical records (including internal sampling data not reported);*
2. *all original recordings for any continuous monitoring instrumentation;*
3. *all instrumentation, calibrations, and maintenance records;*
4. *all plant operation and maintenance records;*
5. *all reports required by this permit; and*
6. *records of all data used to complete the application for the general permit shall be maintained until such time as the system is abandoned*

Inspection

Whether or not the sample collection is done in conjunction with an inspection of the system, obvious malfunctions in the operation of the system must be recorded and reported to the homeowner and local health district. Conditions causing human, pet, or insect exposure to wastewater must be reported to the board of health immediately.

Samples that Exceed Permit Limits

Grab sample results that exceed permit limits may be due to a number of environmental, operational, system, and collection conditions or a combination of these conditions. When local health districts receive testing results they are required to determine compliance with the effluent limits outlined in the general permits. When it is determined that the reported results exceed permit limits the district must report the results to the Ohio EPA. At that time the local health district and Ohio EPA, in cooperation with ODH, must consider the systems operation and possible compliance and enforcement actions. Follow-up system maintenance by a service provider, additional monitoring, and / or the collection of additional samples may be necessary.

The sampling procedures used to collect the results and the homeowners use and operation must also be considered.

Although compliance and enforcement action may be necessary to determine the cause of poor testing results in some situations, it must be noted that grab sample results only reflect performance at the time that the sample was collected, and then only if the sample was collected properly. The function and operation of the individual system or an approved treatment train cannot be predicted through analysis of the results from a single grab sample. This type of analysis can only be conducted while analyzing a sufficient amount of results, collected over time.

For more information on sewage treatment systems, contact the ODH Bureau of Environmental Health at 614-644-1390, BEH@odh.ohio.gov, or visit the ODH Sewage Program Website http://www.odh.ohio.gov/odhPrograms/eh/sewage/sewage1.aspx

References and Resources:

American Public Health Association, American Waterworks Association Water Environmental Federation. *Standard Methods for the Examination of Water and Wastewater, 21st edition.*

Consolidated Treatment Systems, (2008) Sampling Protocol for Multi-Flo Systems.

Norweco, Inc. Effluent Sampling For Residential Treatment Systems.

Ohio EPA. (2009) *Manual of OEPA Surveillance Methods and Quality Assurance Practices.* http://www.epa.state.oh.us/dsw/documents/Field_Manual_1-9-09_revision.pdf

US Environmental Protection Agency. *National Pollutant Discharge Elimination System Compliance Inspection Manual.* http://www.epa.gov/compliance/resources/publications/monitoring/cwa/inspections/npdesinspect/npdesmanual.html

US Environmental Protection Agency. Rules and Regulations 40CFR (1998) Part 136. http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=abc63a76fcb37a07bf241073489c541e&rgn=div5&view=text&node=40:22.0.1.1.1&idno=40

US Environmental Protection Agency. National Pollutant Discharge Elimination System General Permit OHK0001 and OHL0001. http://www.epa.state.oh.us/dsw/permits/GP_HouseholdSewageTreatmentPlants.html#OHK000001

Appendix A

The tables and information provided in this appendix are from the Ohio Environmental Protection Agency (EPA) National Pollutant Discharge Elimination System (NPDES) General Permits OHK000001 and OHL000001 and is provided for the convenience of individuals, companies, and agencies performing the monitoring requirements outlined within them.

Part III. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

Table A.1. FINAL EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS FOR DISCHARGES FROM HSTS DESIGNED IN ACCORDANCE WITH OAC 3701-29, DISCHARGING TO WATERS OTHER THAN LAKE ERIE.

During the period beginning on the effective date of this permit and lasting until the expiration date, the permittee is authorized to discharge in accordance with the following limitations and monitoring requirements.

EFFLUENT CHARACTERISTICS			DISCHARGE LIMITATIONS		MONITORING REQUIREMENTS (3)	
Reporting Code	Units	Parameter	Concentration 30 Day	Daily	Meas. Frequency	Sample Type
00056	GPD	Flow Rate	-	-	1/year	24HrTot.Est.
00530	mg/l	Total Suspended Solids	-	18	1/year	Grab
00610	mg/l	Nitrogen,Ammonia(NH_3)				
		(summer)	-	2.0	1/year	Grab
		(winter)	-	4.5	1/year	Grab
80082	mg/l	$CBOD_5$	-	15	1/year	Grab
31616	#/100ml	Fecal Coliform (summer)	-	2000	1/year	Grab
00083	-	color, severity (1)	-	-	1/year	Estimate
01330	-	odor, severity (1)	-	-	1/year	Estimate
01335	-	turbidity, severity (1)	-	-	1/year	Estimate
00300	mg/l	Dissolved Oxygen	not less than 6.0 at any time		1/year	Grab
50060	mg/l	Chlorine,total residual (2)	not to exceed 0.038 at any time		1/year	Grab

(1) See Part IV, paragraph E.
(2) See Part IV, paragraph F.
(3) Additional operational monitoring requirements shall comply with those listed in OAC 3701-29 for all system components, including service contracts as applicable.

Table A.2. FINAL EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS FOR DISCHARGES FROM HSTS DESIGNED IN ACCORDANCE WITH OAC 3701-29, DISCHARGING DIRECTLY TO LAKE ERIE.

During the period beginning on the effective date of this permit and lasting until the expiration date, the permittee is authorized to discharge in accordance with the following limitations and monitoring requirements.

EFFLUENT CHARACTERISTICS			DISCHARGE LIMITATIONS		MONITORING REQUIREMENTS (3)	
Reporting Code	Units	Parameter	Concentration 30 Day	Daily	Meas. Frequency	Sample Type
00056	GPD	Flow Rate	-	-	1/year	24HrTot.Est.
00530	mg/l	Total Suspended Solids	-	18	1/year	Grab
00610	mg/l	Nitrogen,Ammonia(NH_3)				
		(summer)	-	2.0	1/year	Grab
		(winter)	-	4.5	1/year	Grab
80082	mg/l	$CBOD_5$	-	15	1/year	Grab
31648	#/100ml	*E. coli* (summer)	126	-	1/year	Grab
00083	-	color, severity (1)	-	-	1/year	Estimate
01330	-	odor, severity (1)	-	-	1/year	Estimate
01335	-	turbidity, severity (1)	-	-	1/year	Estimate
00300	mg/l	Dissolved Oxygen	not less than 6.0 at any time		1/year	Grab
50060	mg/l	Chlorine,total residual (2)	not to exceed 0.038 at any time		1/year	Grab

(1) See Part IV, paragraph E.
(2) See Part IV, paragraph F.
(3) Additional operational monitoring requirements shall comply with those listed in OAC 3701-29 for all system components, including service contracts units as applicable.

Appendix B

The sample chain of custody form with instructions and collection checklist with a key point summary in this appendix are provided for the convenience of individuals, companies, and agencies performing the monitoring requirements outlined in the Ohio Environmental Protection Agency (EPA) National Pollutant Discharge Elimination System (NPDES) General Permits OHK000001 and OHL000001.

SAMPLE CHAIN OF CUSTODY RECORD

Homeowner	**Sample Chain of Custody Record**
Address	**for Discharging Household**
Collected by	**Sewage Treatment Systems**
Company name	**Ohio Department of Health**
Date	**2009**

Date	Time	Sample ID	Preservative	Analysis Requested		Remarks

Relinquished by: Name	Company	Signature	Date	Time
Received by: Name	Company	Signature	Date	Time
Relinquished by: Name	Company	Signature	Date	Time
Received by: Name	Company	Signature	Date	Time
Relinquished by: Name	Company	Signature	Date	Time
Received by: Name	Laboratory Name	Signature	Date	Time

SAMPLE CHAIN OF CUSTODY FORM
INSTRUCTIONS

This document ensures that the sample taken in the field is the same sample that is received by the laboratory and provides an explanation of all individuals who handled the sample between its collection and submission to the laboratory. It is therefore important that the form be filled out as accurately and completely as possible.

Homeowner information:
The homeowner, his or her address, and phone number must be printed on the form.
The individual collecting the sample must print his or her name, the company name (if applicable) and the date of the collection.
The individual collecting the sample must also print his or her name, company name (if applicable) signature, date, and time of the collection on the first line labeled:
Relinquished by

Sample Information:

- For each sample, indicate the date and time of the collection.
- When collecting a number of samples for laboratory analysis it may be advantageous to number or letter the samples to avoid confusion. Any identification should be labeled on the sample bottle and on the chain of custody form.
- Under Preservative, indicate the type of preservative in the containers submitted to the lab.
- Include what analyses are to be performed.
- Include any remarks for the laboratory, special instructions, or any necessary documentation related to the sample or its collection.

Verifying Chain of Custody
Each time the sample changes hands, the person relinquishing the sample must print and sign his or her name, company name (if applicable), the date and time the sample was transferred. The person receiving the sample should do likewise.

RECOMMENDED SAMPLING CHECKLIST

Date of Collection: _____ Time of Collection: _____

Site Address: _____

Homeowner: _____

Collectors Name: _____ Company Name:_____

Collectors Signature: _____

Y	N	N/A	
			Sampling frequency in compliance with permit. Date of last sampling: _____
			Proper safety techniques followed.
			Sample collection location is representative of discharge.
			Describe sample collection location: _____
			_____
			Flow is representative of normal household flow: _____
			Flow is induced in system for sampling: _____
			Temperature measured: _____
			Dissolved oxygen measured: _____
			Chlorine measured: _____
			Measures taken to stabilize chlorine: _____
			_____
			Turbidity measured: _____
			Odor measured: _____
			Color measured: _____

For turbidity, odor and color, use the following table to determine the value between 0 and 4 that is reported.

REPORTED VALUE*	SEVERITY DESCRIPTION	TURBIDITY	ODOR	COLOR
0	None	Clear	None	Colorless
1	Mild			
2	Moderate	Light Solids	Musty	Grey
3	Serious			
4	Extreme	Heavy Solids	Septic	Black

* interpolate between the descriptive phrases from OEPA General NPDES Permit OHK00001, OHL00001

			Presence of sludge deposits, fats, oil, grease or other floating material noted: _____
			_____
			Foam, grease, floating solids observed at the outfall.
			Samples refrigerated immediately after collection and during transport.
			All instruments are properly calibrated.
			All chemicals are "fresh."
			Containers and sample holding times conform to 40 CFR 136.3.
			Employ chain of custody procedures. (Chain of Custody is attached)
			Laboratory results attached.

Key points

- **Read and understand the Ohio Department of Health's *Procedure for the Collection and Testing of Grab Samples as Required by NPDES General Permits for Discharging Household Sewage Treatment Systems, all references, and all* manufacturer manuals before implementing a sampling and monitoring program.**
- **Collect all samples in a manner to limit contact with the effluent and ensure your safety. Use proper safety equipment.**
- **Choose a laboratory qualified to perform the testing required by the permit. Determine the laboratory's operating hours and weekend or holiday schedule before collecting samples.**
- **TSS, $CBOD_5$, NH_3, and bacterial concentrations must be determined through the analysis of a correctly collected effluent sample at the qualified laboratory.**
- **Flow rate, dissolved oxygen, color, odor, and turbidity, fats, oil, grease, or other floating material, and chlorine concentrations must be measured or calculated in the field.**
- **All equipment must be calibrated and reagents must be fresh.**
- **Obtain sampling bottles from the qualified laboratory whenever possible. Have extra sampling bottles available in case a sample is contaminated.**
- **Collect all samples from the sample port <u>after</u> final treatment. Do not collect water pooled in the sample port.**
- **Follow manufacturer instructions during times of low or no flow.**
- **Collect samples for laboratory analysis correctly:**
- Mark the sample bottles with the site address, time and date of collection, any additives introduced to the sample, and whether chlorine was detected.
- Do not remove the cap from the sample bottle until you are ready to take the sample.
- Remove the cap from the sample bottle holding the exterior of the cap. Do not touch the rim or mouth of the bottle. Some sample bottles are sealed to ensure they remain sterilized. Be sure to remove the tape completely before collecting a sample. Contamination of samples to be analyzed for fecal coliform or E. coli will affect sample results.
- Carefully fill the sample bottle.
    1. Sample bottles to be analyzed for fecal coliform or E. coli must be filled without rinsing. Leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Do not allow the sample bottle rim to touch the effluent piping or any other source of contamination.
    2. Overflow the airtight sample bottle to be analyzed for $CBOD_5$. The presence of oxygen in the airspace will affect these samples. Do not allow the sample bottle rim to touch the effluent piping or any other source of contamination.
- Recap the sample bottle. The bottle should only be open during the collection of the sample.
- If there is any question whether the sample to be analyzed for fecal coliform or E. coli was contaminated during collection, discard the sample and start over at the first step.
- **Pack the sample in ice for transport. Do not use re-freezable ice packs.**
- **Consider travel time to the laboratory, known laboratory operating hours and required holding times when collecting samples. Do not collect samples when they cannot be analyzed within the required holding times.**
- **Follow chain of custody procedures. Fill out a chain of custody form.**
- **Report the laboratories analysis, all calculations and measurements made in the field, site address, homeowners name, time and date of the collection, collection location, your name, your companies name, laboratory name, analytical techniques or methods used, and instrument maintenance and calibration schedules to the local health department.**