CHAPTER 8
SAMPLE HANDLING PROCEDURES

ESSENTIAL ELEMENTS OF A SAMPLING PLAN

- Goals of the Sampling Plan
- Description of the Facility Generating Sludge
- Data Quality Objectives
- Selecting and Describing Sampling Points
- Sample Collection Procedures
- **Sample Handling Procedures**
- Evaluation of Completeness
- Record-Keeping and Reporting Procedures

A thorough sampling plan documents the procedures employed to preserve and protect the material collected during the sampling event while it is being transported from the field to the laboratory.

Sample Preservation

Preservation refers to sample handling processes aimed at preventing or minimizing chemical or biological activity within the sample after it has been collected. Preservation techniques are different for sludge samples and liquid trip or equipment blank samples. Sludge samples are generally preserved by cooling and maintaining samples at 4° C. Depending on the analytical method, liquid samples may be preserved with the combination of a chemical preservative and chilling to 4° C.

To preserve field and laboratory biosolids samples, cooling to 4° C is (in most cases) the most appropriate method since high-solids sewage sludge cannot be mixed with other preservatives. In addition, laboratory personnel must be notified if chemical preservation is to be done by laboratory staff.

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Sample Holding Times

For all environmental samples, the term “holding time” refers to the maximum amount of time that can pass before a sample is analyzed and still obtain valid results. Specific holding times are listed in the details of the particular analytical method used. Holding times can vary from several hours for microbial analysis to several months or longer for metals analysis. For composite samples, the holding time is assumed to commence when the last portion of sampled material has been obtained.

Samples analyzed beyond the maximum holding time generate questionable or invalid results. Therefore, it is essential that analyses are conducted in a timely manner within the specified holding times.

Appendix D lists the specific analyses required by states in the Northeast. It also includes requirements for sample preservation and holding times. Specific analytical methods should always be consulted well in advance of any sampling effort to confirm adherence to method requirements regarding preservation and holding time. State and federal regulatory staff should also be contacted to ensure that proper methods, containers, and preservatives are employed. In addition, the following tables provide general examples of preservation temperatures and maximum holding times, from field collection to analysis, for typical biosolids sample analyses.

Always refer to specific regulations or applicable permits for holding times when sampling for permit compliance.

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The information in Tables 8-1 and 8-2 is presented to provide general examples only. You should consult the appropriate state and federal regulations and coordinate with the laboratory conducting your analysis to determine specific methods, holding times, preservation, and container requirements that are applicable. This should be done well in advance of any sampling or analysis activities.

Table 8-1. PRESERVATION AND HOLD TIMES FOR ANALYSIS OF BIOSOLIDS SAMPLES

Analyte	Preservation	Maximum Hold Time from Field Collection to Analysis*
Aluminum	Cool to 4° C	6 months
Arsenic	Cool to 4° C	6 months
Barium	Cool to 4° C	6 months
Cadmium	Cool to 4° C	6 months
Chromium	Cool to 4° C	6 months
Copper	Cool to 4° C	6 months
Lead	Cool to 4° C	6 months
Mercury	Cool to 4° C	28 days
Molybdenum	Cool to 4° C	6 months
Nickel	Cool to 4° C	6 months
Selenium	Cool to 4° C	6 months
Silver	Cool to 4° C	6 months

Table 8-1 *continued* **PRESERVATION AND HOLD TIMES FOR ANALYSIS OF BIOSOLIDS SAMPLES**

Analyte	Preservation	Maximum Hold Time from Field Collection to Analysis*
Zinc	Cool to 4° C	6 months
Total Kjeldahl Nitrogen	Cool to 4° C	28 days
Ammonia Nitrogen	Cool to 4° C	28 days
Nitrate Nitrogen	Cool to 4° C	28 days
Total Phosphorus	Cool to 4° C	28 days
Potassium	Cool to 4° C	6 months
Chloride	Cool to 4° C	28 days
Sulfate	Cool to 4° C	28 days
Total Organic Carbon	Cool to 4° C	28 days
Volatile & Semi-volatile Organic Compounds	Cool to 4° C	14 days
Co-planar PCB & Dioxin	Cool to 4° C	1 year
% Total Solids	Cool to 4° C	7 days
% Volatile Solids	Cool to 4° C	7 days
Fecal Coliform	Cool to 4° C	24 hours
Salmonella	Cool to 4° C	24 hours
Enteric Viruses	Cool to between 0 and 10° C	48 hours when between 0 and 10° C
	Freeze sample and hold at -70° C	28 days when cooled to -70° C
Helminth Ova	Cool to between 0 and 10° C	24 hours

** Refer to specific regulations or applicable permits for holding times when sampling for permit compliance.*

Table 8-2. **PRESERVATION AND HOLDING TIMES FOR TCLP ANALYSIS OF BIOSOLIDS SAMPLES**

	Preservation	Maximum Hold Time from Collection to to TCLP Extraction
Organic Compounds	Cool to 4° C	14 days
Mercury	Cool to 4° C	28 days
Metals except Mercury	Cool to 4° C	180 days

Chain of Custody

An integral element of an effective sampling program (documented in a sampling plan) is the use of appropriate chain-of-custody procedures to ensure that laboratory results can be used for compliance, litigation, or enforcement purposes. Chain-of-custody procedures are necessary to ensure the legal integrity of sample materials collected and submitted to a laboratory for analysis. The validity of the test results is enhanced if it can be shown that after the samples were collected they were maintained in a manner that protected them from tampering or interaction with adulterating chemicals. A chain-of-custody form is the written documentation of the security of a sample from the time it is collected to the time it is transferred to the representative of the laboratory that is conducting the analysis. An example of a chain-of-custody form is contained in Appendix J.

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A sample is under your custody if:

- It is in your possession.
- It is in your view, after being in your possession.
- It was in your possession and then locked up to prevent tampering.
- It is in a designated secure area.

Submittal of Samples

Anyone involved in biosolids sampling should, as a standard practice, request chain-of-custody handling of their samples, especially if the results may be used for compliance, enforcement, or other legal purposes. Individuals collecting samples that require chain-of-custody procedures can include regulatory personnel, staff of consulting companies under contract to a regulatory agency, or a biosolids generator/manager taking samples to support a biosolids management program.

Whether samples are hand-carried or delivered by courier to the laboratory, they must be properly preserved, individually sealed, and submitted with a chain-of-custody record. After the laboratory receives the samples, a copy of this record should be returned to the sampling staff. To track samples from collection in the field to receipt of a laboratory report, field sampling personnel may use either a generic or customized chain-of-custody transfer record form.

All too frequently, however, sample containers are not properly protected with custody seals. If you do not seal individual sample containers with custody seals, they will not meet the legal requirements for chain-of-custody protected samples.

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While most laboratories will accept samples that were not maintained and transferred using this procedure, chain-of-custody documentation utilizing custody seals is required in many instances to demonstrate that proper chain-of-custody procedures

were followed in the handling and processing of samples. Samples that are handled and transported without evidence of proper custody may be inadmissible for compliance, enforcement, or other legal purposes.

To further assure the admissibility of biosolids samples for legal purposes, field sampling personnel must observe the following protocols:

- Document in a field notebook all details regarding sampling activities. Documentation must include exact information regarding date, time, location, names of people present, unusual events, field measurements, details of sample storage and security, and transfer of samples to others. If a composite sample is created from multiple grab samples, record the number of grab samples and their relative weighting.
- Use the proper sampling containers, chemicals for sample preservation, coolers, sample labels, chain-of-custody sealing tape, and chain-of-custody record form. Field personnel should also carry with them a listing of acceptable holding times, sampling procedures, and preservation techniques from the laboratory that will conduct the analyses.
- Collect samples according to standard procedures and add a preservative if required. Field personnel needing to break the custody seals at the laboratory to add preservation chemicals should transfer custody to the laboratory after the samples are resealed. Lab personnel must be notified if preservation is to be done at the lab by laboratory staff. The opening and resealing of samples must be documented in field notes and on the chain-of-custody form.

Lab personnel must be notified if preservation is to be done at the lab by laboratory staff.

- After collecting samples, seal the top of each sampling container with a chain-of-custody seal, initial the seal, complete the identifying label, and store and transport the samples in a sealed cooler with ice—if thermal preservation is required. A secure container capable of being sealed is acceptable if thermal preservation is not required. Once all samples are sealed and stored, seal the cooler or container with a custody seal. This seal should be signed by the person who placed the samples inside.
- Use pre-printed, adhesive-backed, chain-of-custody seals (designed for this purpose) on sample containers and coolers. Masking tape, adhesive tape, or common Scotch tape should never be used for chain-of-custody seals because these tapes can be removed without showing evidence of tampering. A failure to use tamper-evident seals may render samples inadmissible for compliance, enforcement, or other legal needs.
- Never leave samples unattended unless they have been locked or secured with initialed custody seals in place.
- Deliver samples to the laboratory supervisor or his/her designee, who will accept the samples and perform the following steps:
  - Verify that correct containers were used and required preservation was performed.
  - Verify that all samples listed on the chain-of-custody record form are accounted for.
  - Verify that all containers are properly sealed, all seals are intact, and the chain-of-custody form and seals are completed correctly.
  - Accept the samples and sign the chain-of-custody record form.
  - Log samples into the laboratory's sample data management system.

– Store samples in a designated locked refrigerator.

– Provide the designated individual with the chain-of-custody record form to file.

Chain-of-Custody Record Form

When using chain-of custody tracking procedures, it is essential that sampling staff complete all items on the chain-of custody record form upon completion of sample collection and prior to submitting the samples to the laboratory. In some jurisdictions, a witness may be required to be present during sampling to satisfy the chain-of-custody requirements of sampling. If a witness is required, they must also sign the form. The form should include the following elements and considerations:

- The project name and number as assigned.
- The sampler(s) (and witness-if required) must sign the form when samples are collected.
- The name of the laboratory performing the analysis.
- The sample location.
- The date and time of sample collection and whether the sample was a composite or grab.
- The description and number of containers, as well as the analyses to be performed.
- The total number of sample containers per location and any remarks regarding the sample.
- The signatures and dates from both parties when custody is transferred from one person to another. If someone other than the person whose signature appears at the top of the form transports the samples to the laboratory, document the transfer of custody on the form.

If the sample is to leave the laboratory for any reason, the sample must be resealed and a chain-of-custody record form reinitiated.

Transportation of Samples

From the time samples are collected, proper handling is critical to sample validity. Exposure to extreme temperatures may compromise collected samples, and testing results may not accurately reflect the true field conditions. Care should be taken to avoid leaving samples in places such as vehicles, postal boxes, and other spaces that could expose them to extreme temperatures. High temperatures can encourage growth of bacteria, which may degrade the organic components in a sample. Freezing is also a concern because it can cause sample containers to break.

Prior to any sample-collection activity, it is very important that the holding times for particular analyses are reviewed and that a suitable method of transporting the samples to the laboratory is selected. For example, samples that have short holding times (e.g., the 6-hour holding time for fecal coliform or salmonella analysis) should not be transported by commercial ground freight service, as they will be received by the laboratory well beyond the allowable holding time. In short, samples must be delivered to the laboratory in the most expedient manner possible following their collection. Note, any material that is identified in the DOT Hazardous Material Table (49 CFR 172.101) must be labeled and transported as prescribed in the table. The three most common methods of delivering samples to the laboratory are:

Hand delivery – This is the most common approach. Unless special arrangements are made, hand delivered samples should be submitted to a laboratory during its normal working hours

and should only be relinquished to the laboratory supervisor or his/her designee.

Overnight-courier or package-delivery service – This method, using commercial carriers such as FedEx or UPS, requires implementation of additional procedures. Thermal preservation must be maintained by packing samples in a sealable cooler with a sufficient volume of ice. (Note: "Blue ice" and most other prepackaged coolants do not cool samples sufficiently.) Unless special arrangements are made, use overnight delivery so that samples arrive before noon during the laboratory's normal workday. It is also highly recommended that sampling events take place early in the week, so samples are received at the laboratory early in their workweek. Samples should not be shipped on a Friday or immediately prior to a holiday unless prior arrangements for receiving the samples have been made with the laboratory.

US Postal Service (USPS) – USPS will not accept for delivery any materials that are classified by the Department of Transportation and postal regulations as hazardous. This restriction precludes USPS delivery of any biosolids samples except for Class A biosolids products. If USPS delivery services are used, the overnight delivery option must be utilized. Be sure that delivery is made directly to the laboratory—not to a centralized "mail room" facility. As with overnight-courier or package-delivery services, samples must be cooled with ice, not with "blue ice" or other prepackaged coolants. Samples should not be shipped on a Friday or immediately prior to a holiday unless prior arrangements for receiving samples have been made with the laboratory.

However samples are delivered, and particularly when using overnight delivery, it is critical that the samples are packed in a manner that ensures that the sample containers are protected from breakage or leakage and that the proper preservation temperature is maintained.

Most laboratories will either reject samples outright or refuse to guarantee their analytical results if samples are received outside of the range of the specified preservation temperature. Generally, samples are required to be chilled to 4° C, with an acceptable range of plus or minus 2° C.

The following is an example of how samples (particularly liquid samples) should be packed for shipping:

Packing Samples for Shipping

1. Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, place an appropriate amount of ice into each of two plastic zipper-lock bags. To prevent leaks, place each ice pack into an additional zipper-lock bag. Seal each zipper-lock bag, expelling as much air as possible, and secure the top with tape.

   Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and zipper-lock bags around ice will prevent leakage and delays.

2. Place a chilled cubitainer—typically a smaller insulated box that fits inside the larger cooler—upright into the center of the lined cooler. Place the two ice packs into the cooler, one on each side of the cubitainer.

3. Place each individual sample container into a zipper-lock bag, expel as much air as possible, seal the bag, and place inside the cubitainer.

4. If you will be monitoring the sample temperature during shipment, place a temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron iButton) inside the cooler. Seal each liner bag by twisting the top of the bag and tying in a knot.

5. Peel the backing off a plastic airbill sleeve and attach the sleeve to the inside of the cooler lid. Alternatively, tape a plastic zipper-lock bag to the lid of the cooler. Sign and date the chain-of-custody form, and place it inside the plastic sleeve or zipper-lock bag on the inside of the cooler lid.

6. Close the cooler lid, seal the horizontal joints with duct or packing tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

   Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

7. Peel the backing off of a second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

8. Ship samples on the day of collection and use a reliable shipping service for next-day delivery. If samples are not shipped on the day of collection, maintain the samples at less than 10° C (but not frozen) by storing in a refrigerator or cooler filled with ice.

9. Notify the laboratory of the sample shipment. Ask the laboratory to contact you to confirm receipt. If the laboratory's receipt of the samples is delayed, use the airbill number to track the sample shipment, utilizing the shipping company's web page or by contacting the shipping company by phone.

At the Laboratory

Once the samples have been received in the laboratory, the supervisor or his/her designee is responsible for their care and custody. That person should be prepared to testify that the samples were in his/her possession or secured in the laboratory at all times—from the moment they were received until the analyses were performed.

CHAPTER 8 REFERENCES:

Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 1992

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Sampling Procedures and Protocols for the National Sewage Sludge Survey. US EPA, Office of Water Regulations and Standards (WH-522), Industrial Technology Division. August 1988.