

1060 COLLECTION AND PRESERVATION OF SAMPLES*

1060 A. Introduction

It is an old axiom that the result of any testing method can be no better than the sample on which it is performed. It is beyond the scope of this publication to specify detailed procedures for the collection of all samples because of varied purposes and analytical procedures. Detailed information is presented in specific methods. This section presents general considerations, applicable primarily to chemical analyses. See appropriate sections for samples to be used in toxicity testing and microbiological, biological, and radiological examinations.

The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and yet large enough for analytical purposes while still accurately representing the material being sampled. This objective implies that the relative proportions or concentrations of all pertinent components will be the same in the samples as in the material being sampled, and that the sample will be handled in such a way that no significant changes in composition occur before the tests are made.

Frequently, the objective of sampling and testing is to demonstrate whether continuing compliance with specific regulatory requirements has been achieved. Samples are presented to the laboratory for specific determinations, with the sampler being responsible for collecting a valid and representative sample. Because of the increasing importance placed on verifying the accuracy and representativeness of data, greater emphasis is placed on proper sample collection, tracking, and preservation techniques. Often, laboratory personnel help plan a sampling program in consultation with the user of the test results. Such consultation is essential to ensure selected samples and analytical methods provide a sound and valid basis for answering the questions that prompted the sampling and that will meet regulatory and/or project-specific requirements.

This section addresses the collection and preservation of water and wastewater samples; the general principles also apply to the sampling of solid or semisolid matrices.

1. General Requirements

Obtain a sample that meets the requirements of the sampling program and handle it so it does not deteriorate or become contaminated or compromised before it is analyzed.

Ensure that all sampling equipment is clean and quality-assured before use. Use sample containers that are clean and free of contaminants. Bake at 450°C all bottles to be used for organic-analysis sampling.

Fill sample containers—without prerinsing—with sample; prerinsing results in loss of any pre-added preservative and sometimes

can bias results high when certain components adhere to the sides of the container. Depending on determinations to be performed, fill the container full (most organic compound determinations) or leave space for aeration, mixing, etc. (microbiological and inorganic analyses). If a bottle already contains preservative, take care not to overfill the bottle, as preservative may be lost or diluted. Except when sampling for analysis of volatile organic compounds or radon, leave an air space equivalent to approximately 1% of the container volume to allow for thermal expansion during shipment.

Special precautions (discussed below) are necessary for samples containing organic compounds and trace metals. Because many constituents may be present at low concentrations (micrograms or nanograms per liter), they may be totally or partially lost or easily contaminated when proper sampling and preservation procedures are not followed.

Composite samples can be obtained by collecting over a period of time, depth, or at many different sampling points. The details of collection vary with local conditions, so specific recommendations are not universally applicable. Sometimes it is more informative to analyze numerous separate samples instead of one composite so variability, maxima, and minima can be determined.

Because of the inherent instability of certain properties and compounds, composite sampling for some analytes is not recommended where quantitative values are desired (examples include oil and grease, acidity, alkalinity, carbon dioxide, chlorine residual, iodine, hexavalent chromium, nitrite, volatile organic compounds, radon-222, dissolved oxygen, ozone, temperature, and pH). In certain cases, such as for BOD, phenolics, sulfite, and cyanide, composite samples are routinely required by regulatory agencies. Refrigerate composite samples for BOD, nitrate, ammonia, TKN, TSS, COD, and TOC.

Sample carefully to ensure that analytical results represent actual sample composition. Important factors affecting results are the presence of suspended matter or turbidity, the method chosen for removing a sample from its container, and the physical and chemical changes brought about by storage or aeration. Detailed procedures are essential when processing (blending, sieving, filtering) samples to be analyzed for trace constituents, especially metals and organic compounds. Some determinations can be invalidated by contamination during processing. Treat each sample individually with regard to the substances to be determined, the amount and nature of turbidity present, and other conditions that may influence the results.

Carefully consider the technique for collecting a representative sample and define it in the sampling plan. For metals, it often is appropriate to collect both a filtered and an unfiltered sample to differentiate between total and dissolved metals present in the matrix. Be aware that some metals may partially sorb to filters. Beforehand, determine the acid requirements to bring the pH to <2 on a separate sample. Add the same relative amount of acid to all samples; use ultrapure acid preservative to prevent contamination. Be sure that the dilution caused by acidifying is negligible or sufficiently reproducible for a dilution correction factor. When

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2011 revisions by L. Malcom Baker, Rodger B. Baird, Nilda B. Cox, Andrew D. Eaton.
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filtered samples will be collected, filter them in the field, if possible, or at the point of collection before preservation with acid. Filter samples in a laboratory-controlled environment if field conditions could cause error or contamination; in this case, filter as soon as possible. Often, slight turbidity can be tolerated if experience shows that it will cause no interference in gravimetric or volumetric tests and that its influence can be corrected in colorimetric tests, where it has potentially the greatest interfering effect. Sample collector must state whether the sample has been filtered.

Make a record of every sample collected and identify every bottle with a unique sample number, preferably by attaching an appropriately inscribed tag or label. Document sufficient information to provide positive sample identification at a later date, including the unique sample identification number, the name of the sample collector, the date, hour, exact location, and, if possible, sample type (e.g., grab or composite) and any other data that may be needed for correlation, such as water temperature, weather conditions, water level, stream flow, and post-collection conditions. If all pertinent information will not fit on a label or attached tag, record information in a bound sample log book at the sampling site at the time of sample collection. Use waterproof ink to record all information (preferably with black, non-solvent-based ink). Fix sampling points by detailed description in the sampling plan, by maps, or with the aid of stakes, buoys, or landmarks in a manner that will permit their identification by other persons without reliance on memory or personal guidance. Global positioning systems (GPS) also can supply accurate sampling position data. Particularly when sample results are expected to be involved in litigation, use formal "chain-of-custody" procedures (see 1060B.2), which trace sample history from collection to final reporting.

Before collecting samples from distribution systems, flush lines with three to five pipe volumes (or until water is being drawn from the main source) to ensure that the sample is representative of the supply, taking into account the volume of pipe to be flushed and the flow velocity. If the distribution system volume is unavailable, flush with tap fully open for at least 2 to 3 min before sampling. An exception to these guidelines (i.e., collecting a first-draw sample) is when information on areas of reduced or restricted flow is desired or when samples for lead in drinking water are being collected.

Although well-pumping protocols depend on the objectives of an investigation and other factors, such as well characteristics and available equipment, a general rule is to collect samples from wells only after the well has been purged sufficiently (usually with three to ten well volumes) to ensure that the sample represents the groundwater. Purging stagnant water is critical. Sometimes it will be necessary to pump at a specified rate to achieve a characteristic drawdown, if this determines the zones from which the well is supplied; record purging rate and drawdown, if necessary. By using methods with minimal drawdown, purging volumes can be reduced significantly.

When samples are collected from a river or stream, observed results may vary with depth, stream flow, and distance from each shore. Selection of the number and distribution of sites at which samples should be collected depends on study objectives, stream characteristics, available equipment, and other factors. If equipment is available, take an integrated sample from top to bottom in the middle of the main channel of the stream or from side to

side at mid-depth. If only grab or catch samples can be collected, preferably take them at various points of equal distance across the stream; if only one sample can be collected, take it in the middle of the main channel of the stream and at mid-depth. Integrated samples are described further in 1060B.1c.

Rivers, streams, lakes, and reservoirs are subject to considerable variations from normal causes (e.g., seasonal stratification, diurnal variations, rainfall, runoff, and wind). Choose location, depth, and frequency of sampling depending on local conditions and the purpose of the investigation.

Use the following examples for general guidance. Avoid areas of excessive turbulence because of potential loss of volatile constituents and potential presence of denser-than-air toxic vapors. Avoid sampling at weirs, if possible, because such locations tend to favor retrieval of lighter-than-water immiscible compounds. Generally, collect samples beneath the surface in quiescent areas and open sampling container below surface with the mouth directed toward the current to avoid collecting surface scum unless oil and grease is a constituent of interest; then collect water at the surface. If composite samples are required, ensure that sample constituents are not lost during compositing because of improper handling of portions being composited. If samples will be analyzed for organic constituents, refrigerate composited portions. Do not composite samples for VOC analysis because some of the components will be lost through volatilization.

2. Safety Considerations

Because sample constituents may be toxic, take adequate precautions during sampling and sample handling. Toxic substances can enter through the skin and eyes and, in the case of vapors, also through the lungs. Ingestion can occur via direct contact of toxic materials with foods or by adsorption of vapors onto foods. Precautions may be limited to wearing gloves or may include coveralls, aprons, or other protective apparel. Often, the degree of protection provided by chemical protective clothing (CPC) is specific for different manufacturers and their product models¹; ensure that the clothing chosen will offer adequate protection. Always wear eye protection (e.g., safety glasses with side shields or goggles). When toxic vapors may be present, sample only in well-ventilated areas, or use an appropriate respirator or self-contained breathing apparatus. In a laboratory, open sample containers in a fume hood. Never have food in the laboratory, near samples, or near sampling locations; always wash hands thoroughly before handling food.²

Always prohibit eating, drinking, or smoking near samples, sampling locations, and in the laboratory. Keep sparks, flames, and excessive heat sources away from samples and sampling locations. If flammable compounds are suspected or known to be present and samples will be refrigerated, use only specially designed explosion-proof refrigerators.²

Collect samples safely, avoiding situations that may lead to accidents. When in doubt as to the level of safety precautions needed, consult a knowledgeable industrial hygienist or safety professional. Samples with radioactive contaminants may require other safety considerations; consult a health physicist.

Label adequately any sample known or suspected to be hazardous because of flammability, corrosivity, toxicity, oxidizing chemicals, or radioactivity, so appropriate precautions can be taken during sample handling, storage, and disposal.

3. References

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1060 B. Collection of Samples

1. Types of Samples

a. Grab samples: Grab samples are single samples collected at a specific spot at a site over a short period of time (typically seconds or minutes). Thus, they represent a “snapshot” in both space and time of a sampling area. Discrete grab samples are taken at a selected location, depth, and time. Depth-integrated grab samples are collected over a predetermined part or the entire depth of a water column, at a selected location and time in a given body of water.

A sample can represent only the composition of its source at the time and place of collection. However, when a source is known to be relatively constant in composition over an extended time or over substantial distances in all directions, then the sample may represent a longer time period and/or a larger volume than the specific time and place at which it was collected. In such circumstances, a source may be represented adequately by single grab samples. Examples are protected groundwater supplies, water supplies receiving conventional treatment, some well-mixed surface waters, but rarely wastewater streams, rivers, large lakes, shorelines, estuaries, and groundwater plumes.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes, which may vary from 5 min to 1 h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space (i.e., from location to location) rather than time, collect samples from appropriate locations that will meet the objectives of the study (for example, upstream and downstream from a point source).

The same principles apply to sampling wastewater sludges, sludge banks, and muds, although these matrices are not specifically addressed in this section. Take every possible precaution to obtain a representative sample or one conforming to a sampling program.

b. Composite samples: Composite samples should provide a more representative sampling of heterogeneous matrices in which the concentration of the analytes of interest may vary over short periods of time and/or space. Composite samples can be obtained by combining portions of multiple grab samples or by using specially designed automatic sampling devices. Sequential (time) composite samples are collected by using continuous, constant sample pumping or by mixing equal water volumes collected at regular time intervals. Flow-proportional composites are collected by continuous pumping at a rate proportional to the flow, by mixing equal volumes of water collected at time intervals that are inversely proportional to the volume of flow, or by mixing volumes of water proportional to the flow collected during or at regular time intervals.

Advantages of composite samples include reduced costs of analyzing a large number of samples, more representative samples of heterogeneous matrices, and larger sample sizes when amounts of test samples are limited. Disadvantages of composite samples include loss of analyte relationships in individual samples, potential dilution of analytes below detection levels, increased potential analytical interferences, and increased possibility of analyte interactions. In addition, use of composite samples may reduce the number of samples analyzed below the required statistical need for specified data quality objectives or project-specific objectives.

Do not use composite samples with components or characteristics subject to significant and unavoidable changes during storage. Analyze individual samples as soon as possible after collection and preferably at the sampling point. Examples are dissolved gases, residual chlorine, soluble sulfide, temperature, and pH. Changes in components, such as dissolved oxygen or carbon dioxide, pH, or temperature, may produce secondary changes in certain inorganic constituents, such as iron, manganese, alkalinity, or hardness. Some organic analytes also may be changed by changes in the foregoing components. Use time-composite samples only for determining components that can be demonstrated to remain unchanged under the conditions of sample collection, preservation, and storage.

Collect individual portions in a wide-mouth bottle every hour (in some cases, every half hour or even every 5 min) and mix at the end of the sampling period or combine in a single bottle as collected. If preservatives are used, add them to the sample bottle initially so all portions of the composite are preserved as soon as collected.

Automatic sampling devices are available; however, do not use them unless the sample is preserved as described below. Composite samplers running for extended periods (weeks to months) should undergo routine cleaning of containers and sample lines to minimize sample growth and deposits.

c. Integrated (discharge-weighted) samples: For certain purposes, the information needed is best provided by analyzing mixtures of grab samples collected from different points simultaneously, or as nearly so as possible, using discharge-weighted methods [e.g., equal-width increment (EWI) or equal discharge-increment (EDI) procedures and equipment]. An example of the need for integrated sampling occurs in a river or stream that varies in composition across its width and depth. To evaluate average composition or total loading, use a mixture of samples representing various points in the cross-section, in proportion to their relative flows. The need for integrated samples also may exist if combined treatment is proposed for several separate wastewater streams, the interaction of which may have a significant effect on treatability or even on composition. Mathematical prediction of the interactions among chemical components may

be inaccurate or impossible, and testing a suitable integrated sample may provide more useful information.

Both lakes and reservoirs show spatial variations of composition (depth and horizontal location). However, there are conditions under which neither total nor average results are especially useful, but local variations are more important. In such cases, examine samples separately (i.e., do not integrate them).

Preparation of integrated samples usually requires equipment designed to collect a sample water uniformly across the depth profile. Knowledge of the volume, movement, and composition of the various parts of the water being sampled usually is required. Collecting integrated samples is a complicated and specialized process that must be described adequately in a sampling plan.

2. Chain-of-Custody Procedures

Properly designed and executed chain-of-custody forms will ensure sample integrity from collection to data reporting. This includes the ability to trace possession and handling of the sample from the time of collection through analysis and final disposition. This process is referred to as *chain of custody* and is required to demonstrate sample control when the data are to be used for regulation or litigation. Where litigation is not involved, chain-of-custody procedures are useful for routine control of samples.

A sample is considered to be under a person's custody if it is in the individual's physical possession, in the individual's sight, secured and tamper-proofed by that individual, or secured in an area restricted to authorized personnel. The following procedures summarize the major aspects of chain of custody. More detailed discussions are available.^{1,2}

a. Sample labels (including bar-code labels): Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. Include at least the following information: a unique sample number, sample type, name of collector, date and time of collection, place of collection, and sample preservative. Also include date and time of preservation for comparison to date and time of collection. Affix tags or self-adhesive labels to sample containers before, or at the time of, sample collection.

b. Sample seals: Use sample seals to detect unauthorized tampering with samples up to the time of analysis. Use self-adhesive paper seals that include at least the following information: sample number (identical with number on sample label), collector's name, and date and time of sampling. Plastic shrink seals also may be used.

Attach seal so that it must be broken to open the sample container or the sample shipping container (e.g., a cooler). Affix seal to container before sample leaves custody of sampling personnel.

c. Field log book: Record all information pertinent to a field survey or sampling in a bound log book. As a minimum, include the following in the log book: purpose of sampling; location of sampling point; name and address of field contact; producer of material being sampled and address (if different from location); type of sample; and method, date, and time of preservation. If the sample is wastewater, identify process producing waste stream. Also provide suspected sample composition, including concentrations; number and volume of sample(s) taken; description of sampling point and sampling method; date and time of collection; collector's sample identification number(s); sample distribution and how transported; references (e.g., maps or photographs of the sampling site); field

observations and measurements; and signatures of personnel responsible for observations. Because sampling situations vary widely, it is essential to record sufficient information so one could reconstruct the sampling event without reliance on the collector's memory. Protect log book and keep it in a safe place.

d. Chain-of-custody record: Fill out a chain-of-custody record to accompany each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time, and address of collection; sample type; sample preservation requirements; signatures of persons involved in the chain of possession; and inclusive dates and times of possession.

e. Sample analysis request sheet: The sample analysis request sheet accompanies samples to the laboratory. The collector completes the field portion of the form, which includes most of the pertinent information noted in the log book. The laboratory portion of the form is to be completed by laboratory personnel and includes: name of person receiving the sample, laboratory sample number, date of sample receipt, condition of each sample (if it is cold or warm, whether the container is full or not, color, if more than one phase is present, etc.), and determinations to be performed.

f. Sample delivery to the laboratory: Deliver sample(s) to laboratory as soon as practicable after collection, typically within 2 d. If shorter sample holding times are required, make special arrangements to ensure timely delivery to the laboratory. If samples are shipped by a commercial carrier, include the waybill number in the sample custody documentation. Ensure that samples are accompanied by a completed chain-of-custody record and a sample analysis request sheet. Deliver sample to sample custodian.

g. Receipt and logging of sample: In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain-of-custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and/or computerized laboratory information management system, and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is assigned to an analyst.

h. Assignment of sample for analysis: The laboratory supervisor usually assigns the sample for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.

i. Disposal: Hold samples for the prescribed amount of time for the project or until the data have been reviewed and accepted. Document the disposition of samples. Ensure that disposal is in accordance with local-, state-, and U.S. EPA-approved methods.

3. Sampling Methods

a. Manual sampling: Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs. It requires trained field technicians and is often necessary for regulatory and research investigations for which critical appraisal of field conditions and complex sample-collection techniques are essential. Manually collect certain samples, such as waters containing oil and grease.

b. Automatic sampling: Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may

provide the means for more frequent sampling,³ and are increasingly used. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts or that can be contaminated (e.g., from phthalate esters) by contact with them. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential incompatibility of plastic components.

Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

c. Sorbent sampling: Use of solid sorbents, particularly membrane-type disks, is becoming more frequent. These methods offer rapid, inexpensive sampling if the analytes of interest can be adsorbed and desorbed efficiently and the water matrix is free of particulates that plug the sorbent.

4. Sample Containers

The type of sample container used is of utmost importance. Test sample containers and document that they are free of analytes of interest, especially when sampling and analyzing for very low analyte levels. Containers typically are made of plastic or glass, but one material may be preferred over the other. For example, silica, sodium, and boron may be leached from soft glass, but not plastic, and trace levels of some pesticides and metals may sorb onto the walls of glass containers.⁴ Thus, hard glass containers* are preferred. For samples containing organic compounds, do not use plastic containers except those made of fluorinated polymers, such as polytetrafluoroethylene (PTFE).³

Some sample analytes may dissolve (be absorbed) into the walls of plastic containers; similarly, contaminants from plastic containers may leach into samples. Avoid plastics wherever possible because of potential contamination from phthalate esters. Container failure due to breakdown of the plastic is possible. Therefore, use glass containers for all organics analyses, such as volatile organics, semivolatile organics, pesticides, PCBs, and oil and grease. Some analytes (e.g., bromine-containing compounds and some pesticides, and polynuclear aromatic compounds) are light-sensitive; collect them in amber-colored glass containers to minimize photodegradation. Container caps, typically plastic, also can be a problem. Do not use caps with paper liners. Use foil or PTFE liners but be aware that metal liners can contaminate samples collected for metals analysis and they may also react with the sample if it is acidic or alkaline. Serum vials with PTFE-lined rubber or plastic septa are useful.

In rare situations, it may be necessary to use sample containers not specifically prepared for use, or otherwise unsuitable for the particular situation; thoroughly document these deviations. Documentation should include type and source of container, and the preparation technique (e.g., acid washed with reagent water rinse). For QA purposes, the inclusion of a bottle blank may be necessary.

5. Number of Samples

Because of variability from analytical and sampling procedures (i.e., population variability), a single sample is insufficient to reach any reasonable desired level of confidence. If an overall standard

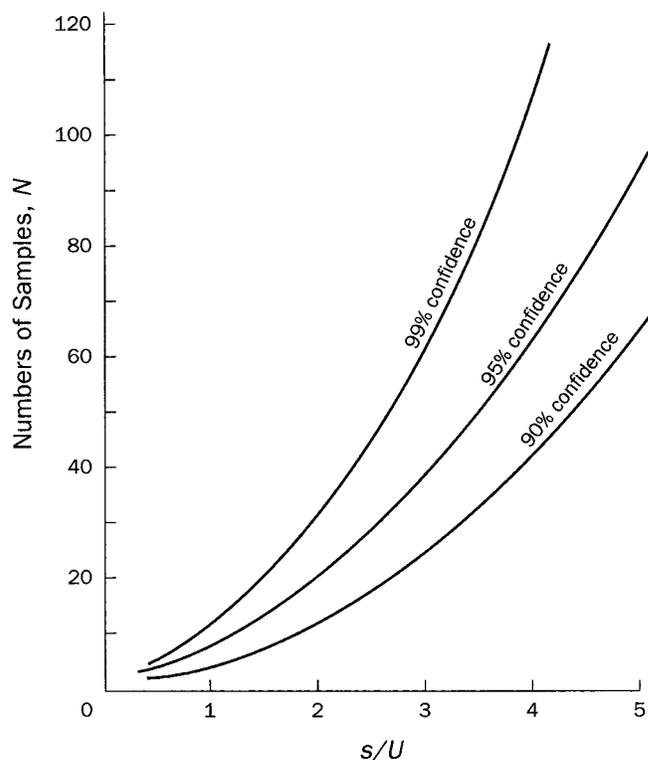


Figure 1060:1. Approximate number of samples required in estimating a mean concentration. SOURCE: Methods for the Examination of Waters and Associated Materials: General Principles of Sampling and Accuracy of Results. 1980. Her Majesty's Stationery Off., London, England.

deviation (i.e., the standard deviation of combined sampling and analysis) is known, the required number of samples for a mobile matrix, such as water, may be estimated as follows:⁴

$$N \geq \left(\frac{ts}{U} \right)^2$$

where:

- N = number of samples,
- t = Student's t statistic for a given confidence level,
- s = overall standard deviation, and
- U = acceptable level of uncertainty.

To assist in calculations, use curves such as those in Figure 1060:1. As an example, if s is 0.5 mg/L, U is ± 0.2 mg/L, and a 95% confidence level is desired, approximately 25 to 30 samples must be taken.

The above equation assumes that total error (population variability) is known. Total variability consists of all sources of variability, including the distribution of the analytes of interest within the sampling site; collection, preservation, preparation, and analysis of samples; and data handling and reporting. In simpler terms, error (variability) can be divided into sampling and analysis components. Sampling error due to population variability (including heterogeneous distribution of analytes in the environmental matrix) usually is much larger than analytical error components. Unfortunately, sampling error usually is not

* Pyrex, or equivalent.

available and the analyst is left with only the published error of the measurement system (typically obtained by using a reagent water matrix under the best analytical conditions).

More accurate equations are available.⁵ These are based on the Z distribution for determining the number of samples needed to estimate a mean concentration when variability is estimated in absolute terms using the standard deviation. The coefficient of variation [relative standard deviation (RSD)] is used when variability is estimated in relative terms.

The number of random samples to be collected at a site can be influenced partly by the method that will be used. The values for standard deviation (SD) or RSD may be obtained from each of the methods or in the literature.⁶ However, calculations of estimated numbers of samples needed based only on this information will result in underestimated numbers of samples because only the analytical variances are considered, and the typically larger variances from the sampling operations are not included. Preferably, determine and use SDs or RSDs from overall sampling and analysis operations.

For estimates of numbers of samples needed for systematic sampling (e.g., drilling wells for sampling groundwater or for systematically sampling large water bodies, such as lakes), equations are available⁷ that relate number of samples to shape of grid, area covered, and space between nodes of grid. The grid spacing is a complex calculation that depends on the size and shape of any contaminated spot (such as a groundwater plume) to be identified, in addition to the geometric shape of the sampling grid.

See individual methods for types and numbers of quality assurance (QA) and quality control (QC) samples [e.g., for normal-level (procedural) or low-level (contamination) bias or for precision] involving sampling or laboratory analysis (either overall or individually). Estimates of numbers of QC samples needed to achieve specified confidence levels also can be calculated. Rates of false positives (Type I error) and false negatives (Type II error) are useful parameters for estimating required numbers of QC samples. A false positive is the incorrect conclusion that an analyte is present when it is absent. A false negative is the incorrect conclusion that an analyte is absent when it is present. If the frequency of false positives or false negatives desired to be detected is <10%, then

$$n = \frac{\ln \alpha}{\ln (1 - Y)}$$

where:

α = (1 - desired confidence level), and
 Y = frequency to detect (<10%).

If the frequency that is desirable to detect is >10%, iterative solution of a binomial equation is necessary.^{5,8}

Equations are available as a computer program† for computing sample number by the Z distribution, for estimating samples needed in systematic sampling, and for estimating required number of QC samples.

6. Sample Volumes

Collect a 1-L sample for most physical and chemical analyses. For certain determinations, larger samples may be necessary. Table 1060:I lists volumes ordinarily required for analyses, but it is strongly recommended that the laboratory that will conduct the analyses also be consulted to verify the analytical needs of sampling procedures as they pertain to the goals and data quality objective of an investigation.

Do not use samples from the same container for multiple testing requirements (e.g., organic, inorganic, radiological, bacteriological, and microscopic examinations) because methods of collecting and handling are different for each type of test. Always collect enough sample volume in the appropriate container in order to comply with sample handling, storage, and preservation requirements.

7. References

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9. U.S. ENVIRONMENTAL PROTECTION AGENCY. 2007. 40 CFR Part 136, Table II.
10. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1992. Rules and Regulations. 40 CFR Parts 100–149.

† DQO-PRO, available (free) by downloading from Instant Reference Sources, Inc., <http://instantref.com/dqo-form.htm>.

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TABLE 1060:I. SUMMARY OF SPECIAL SAMPLING AND HANDLING REQUIREMENTS*

Determination	Container†	Minimum Sample Size mL	Sample Type‡	Preservation§	Maximum Storage Recommended	Regulatory
Acidity	P, G(B), FP	100	g	Cool, $\leq 6^{\circ}\text{C}$	24 h	14 d
Alkalinity	P, G, FP	200	g	Cool, $\leq 6^{\circ}\text{C}$	24 h	14 d
BOD	P, G, FP	1000	g, c	Cool, $\leq 6^{\circ}\text{C}$	6 h	48 h
Boron	F, P (PTFE) or quartz	1000	g, c	HNO_3 to $\text{pH} < 2$	28 d	6 months
Bromide	P, G, FP	100	g, c	None required	28 d	28 d
Carbon, organic, total	G(B), P, FP	100	g, c	Analyze immediately, or cool $\leq 6^{\circ}\text{C}$ and add HCl , H_3PO_4 , or H_2SO_4 to pH	7 d	28 d
Carbon dioxide	P, G	100	g	Analyze immediately	0.25 h	N.S.
COD	P, G, FP	100	g, c	Analyze as soon as possible, or add H_2SO_4 to $\text{pH} < 2$; Cool, $\leq 6^{\circ}\text{C}$	7 d	28 d
Chloride	P, G, FP	50	g, c	None required	N.S.	28 d
Chlorine, total, residual	P, G	500	g	Analyze immediately	0.25 h	0.25 h
Chlorine dioxide	P, G	500	g	Analyze immediately	0.25 h	N.S.
Chlorophyll	P, G	500	g	Unfiltered, dark, $\leq 6^{\circ}\text{C}$ Filtered, dark, -20°C (Do not store in frost-free freezer)	24–48 h 28 d	N.S.
Color	P, G, FP	500	g, c	Cool, $\leq 6^{\circ}\text{C}$	24 h	48 h
Specific conductance	P, G, FP	500	g, c	Cool, $\leq 6^{\circ}\text{C}$	28 d	28 d
Cyanide Total	P, G, FP	1000	g, c	Analyze within 15 min. Add NaOH to $\text{pH} > 12$ if sample is to be stored, Cool, $\leq 6^{\circ}\text{C}$, in dark. Add thiosulfate if residual chlorine present	24 h	14 d; 24 h if sulfide present
Amenable to chlorination	P, G, FP	1000	g, c	Remove residual chlorine with thiosulfate and cool $\leq 6^{\circ}\text{C}$	stat	14 d; 24 h if sulfide present
Fluoride	P	100	g, c	None required	28 d	28 d
Hardness	P, G, FP	100	g, c	Add HNO_3 or H_2SO_4 to $\text{pH} < 2$	6 months	6 months
Iodine	P, G	500	g	Analyze immediately	0.25 h	N.S.
Metals	P(A), G(A), FP (A)	1000	g, c	For dissolved metals filter immediately, add HNO_3 to $\text{pH} < 2$	6 months	6 months
Chromium VI	P(A), G(A), FP (A)	250	g	Cool, $\leq 6^{\circ}\text{C}$, pH 9.3–9.7, ammonium sulfate buffer preservative as specified in method 3500-Cr to extend to 28 d HT	28 d	28 d
Copper by colorimetry	—*	—	g, c	—	—	—
Mercury	P(A), G(A), FP(A)	500	g, c	Add HNO_3 to $\text{pH} < 2$, Cool $\leq 6^{\circ}\text{C}$	28 d	28 d
Nitrogen Ammonia	P, G, FP	500	g, c	Analyze as soon as possible or add H_2SO_4 to $\text{pH} < 2$, Cool, $\leq 6^{\circ}\text{C}$	7 d	28 d
Nitrate	P, G, FP	100	g, c	Analyze as soon as possible; Cool, $\leq 6^{\circ}\text{C}$	48 h	48 h (14 d for chlorinated samples)
Nitrate + nitrite	P, G, FP	200	g, c	Add H_2SO_4 to $\text{pH} < 2$, Cool, $\leq 6^{\circ}\text{C}$	1–2 d	28 d
Nitrite	P, G, FP	100	g, c	Analyze as soon as possible; Cool, $\leq 6^{\circ}\text{C}$	none	48 h
Organic, Kjeldahl	P, G, FP	500	g, c	Cool, $\leq 6^{\circ}\text{C}$, add H_2SO_4 to $\text{pH} < 2$	7 d	28 d
Odor	G	500	g	Analyze as soon as possible; Cool $\leq 6^{\circ}\text{C}$	6 h	24 h (EPA Manual drinking water)
Oil and grease	G, wide-mouth calibrated	1000	g	Add HCl or H_2SO_4 to $\text{pH} < 2$, Cool, $\leq 6^{\circ}\text{C}$	28 d	28 d

COLLECTION AND PRESERVATION OF SAMPLES (1060)/Collection of Samples

TABLE 1060:I. CONT.

Determination	Container†	Minimum Sample Size mL	Sample Type‡	Preservation§	Maximum Storage Recommended	Regulatory
Organic Compounds						
MBAS	P, G, FP	250	g, c	Cool, ≤6°C	48 h	48 h as per CFR 136
Pesticides*	G(S), PTFE-lined cap	1000	g, c	Cool, ≤6°C, add 1000 mg ascorbic acid/L if residual chlorine present (0.008% sodium thiosulfate in CFR 136)	7 d	7 d until extraction; 40 d after extraction
Phenols	P, G, PTFE-lined cap	500	g, c	Cool, ≤6°C, add H ₂ SO ₄ to pH<2	*	28 d until extraction, 2 d after extraction
Purgeables* by purge and trap	G, PTFE-lined cap	2×40	g	Cool, ≤6°C; add HCl to pH<2; add 1000 mg ascorbic acid/L if residual chlorine present (0.008% sodium thiosulfate in CFR 136)	7 d	14 d
Base/neutrals & acids	G(S) amber	1000	g, c	Cool, ≤6°C, 0.008% sodium thiosulfate in CFR 136 if chlorine is present	7 d	7 d until extraction; 40 d after extraction
Oxygen, dissolved	G, BOD bottle	300	g	Analyze immediately	0.25 h	0.25 h
Electrode				Titration may be delayed after acidification	8 h	8 h
Winkler						
Ozone	G	1000	g	Analyze immediately	0.25 h	N.S.
pH	P, G	50	g	Analyze immediately	0.25 h	0.25 h
Phosphate	G(A)	100	g	For dissolved phosphate filter immediately; Cool, ≤6°C	48 h	48 h as per EPA manual for DW
Phosphorus, total	P, G, FP	100	g, c	Add H ₂ SO ₄ to pH<2 and cool, ≤6°C	28 d	28 d
Salinity	G, wax seal	240	g	Analyze immediately or use wax seal	6 months	N.S.
Silica	F, P (PTFE) or quartz	200	g, c	Cool, ≤6°C, do not freeze	28 d	28 d
Sludge digester gas	G, gas bottle	—	g	—	N.S.	
Solids ⁹	P, G	200	g, c	Cool, ≤6°C	7 d	2–7 d; see cited reference
Sulfate	P, G, FP	100	g, c	Cool, ≤6°C	28 d	28 d
Sulfide	P, G, FP	100	g, c	Cool, ≤6°C; add 4 drops 2N zinc acetate/100 mL; add NaOH to pH >9	28 d	7 d
Temperature	P, G, FP	—	g	Analyze immediately	0.25 h	0.25 h
Turbidity	P, G, FP	100	g, c	Analyze same day; store in dark up to 24 h, Cool, ≤6°C	24 h	48 h

* For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible.

† P = plastic (polyethylene or equivalent); G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO₃; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents or baked; FP = fluoropolymer [polytetrafluoroethylene (PTFE, Teflon) or other fluoropolymer].

‡ g = grab; c = composite.

§ Cool = storage at, >0°C, ≤6°C (above freezing point of water); in the dark; analyze immediately = analyze usually within 15 min of sample collection.

|| See citation¹⁰ for possible differences regarding container and preservation requirements. N.S. = not stated in cited reference; stat = no storage allowed; analyze immediately (within 15 min).

Some drinking water (DW) and treated wastewater (WW) matrices may be subject to positive interference as a result of preservation. If such interference is demonstrable, samples should be analyzed as soon as possible without preservation. Do not hold for more than 15 min without demonstrating that cyanide (CN) is stable for longer periods in a specific matrix.

NOTE: This table is intended for guidance only. If there is a discrepancy between this table and the method, the information in the current method takes precedence. If performing the method for compliance purposes, be aware that alternative preservation and holding-time requirements may exist. If so, the regulatory requirements should be used.

1060 C. Sample Storage and Preservation

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, or natural waters, is a practical impossibility because complete stability for every constituent never can be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection.

1. Sample Storage before Analysis

a. Nature of sample changes: Some determinations are more affected by sample storage than others. Certain cations are subject to loss by adsorption to, or ion exchange with, the walls of glass containers. These include aluminum, cadmium, chromium, copper, iron, lead, manganese, silver, and zinc, which are best collected in a separate clean bottle and acidified with nitric acid to a pH <2.0 to minimize precipitation and adsorption on container walls. Also, some organics may be subject to loss by adsorption to the walls of glass containers.

Temperature changes quickly; pH may change significantly in a matter of minutes; dissolved gases (oxygen, carbon dioxide) may be lost. Because changes in such basic water quality properties may occur so quickly, determine temperature, reduction–oxidation potential, and dissolved gases *in situ* and pH, specific conductance, turbidity, and alkalinity immediately after sample collection. Many organic compounds are sensitive to changes in pH and/or temperature resulting in reduced concentrations during storage.

Changes in the pH–alkalinity–carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values for calcium and total hardness.

Iron and manganese are readily soluble in their lower oxidation states but relatively insoluble in their higher oxidation states; therefore, these cations may precipitate or they may dissolve from a sediment, depending on the redox potential of the sample. Microbiological activity may affect the nitrate–nitrite–ammonia content, phenol or BOD concentration, or the reduction of sulfate to sulfide. Residual chlorine is reduced to chloride. Sulfide, sulfite, ferrous iron, iodide, and cyanide may be lost through oxidation. Color, odor, and turbidity may increase, decrease, or change in quality. Sodium, silica, and boron may be leached from the glass container. Hexavalent chromium may be reduced to trivalent chromium.

The biological activity in a sample may change the oxidation state of some constituents. Soluble constituents may be converted to organically bound materials in cell structures, or cell lysis may result in release of cellular material into solution. The well-known nitrogen and phosphorus cycles are examples of biological influences on sample composition.

Zero headspace is important in preservation of samples with volatile organic compounds and radon. Avoid loss of volatile materials by collecting sample in a completely filled container. Achieve this by carefully filling the bottle so top of meniscus is above the top of the bottle rim. It is important to avoid spillage or air entrapment if preservatives, such as HCl or ascorbic acid, have already been added to the bottle. After capping or sealing bottle, check for air bubbles by inverting and gently tapping it; if one or more air bubbles are observed then, if practical, discard

the sample and repeat refilling bottle with new sample until no air bubbles are observed (this cannot be done if bottle contained preservatives before it was filled).

Serum vials with septum caps are particularly useful in that a sample portion for analysis can be taken through the cap by using a syringe,¹ although the effect of pressure reduction in the headspace must be considered. Pulling a sample into a syringe under vacuum can result in low bias data for volatile compounds and the resulting headspace precludes taking further subsamples.

b. Time interval between collection and analysis: In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. For certain constituents and physical values, immediate analysis in the field is required. For composited samples, it is common practice to use the time at the end of composite collection as the sample-collection time.

Check with the analyzing laboratory to determine how much elapsed time may be allowed between sample collection and analysis; this depends on the character of the sample and the stability of the target analytes under storage conditions. Many regulatory methods limit the elapsed time between sample collection and analysis (see Table 1060:I). Changes caused by growth of microorganisms are greatly retarded by keeping the sample at a low temperature (<6°C but above freezing). When the interval between sample collection and analysis is long enough to produce changes in either the concentration or physical state of the constituent to be measured, follow the preservation practices given in Table 1060:I. Record time elapsed between sampling and analysis, and which preservative, if any, was added.

2. Preservation Techniques

To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Dry ice also may effect a pH change in samples. Keep composite samples cool with ice or a refrigeration system set at ≤6°C during compositing. Analyze samples as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, preferably store at ≤6°C.¹

No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations to be made. Use chemical preservatives only when they do not interfere with the analysis being made. When they are used, add them to the sample bottle initially so all sample portions are preserved as soon as collected. Because a preservation method for one determination may interfere with another one, samples for multiple determinations may need to be split and preserved separately. All preservation methods may be inadequate when applied to suspended matter. Do not use formaldehyde as a preservative for samples collected for chemical analysis because it affects many of the target analytes.

Preservation methods are relatively limited and are intended generally to retard biological action, retard hydrolysis of chemical compounds and complexes, and reduce volatility of constituents.

Preservation methods are limited to pH control, chemical addition, the use of amber and opaque bottles, refrigeration, filtration, and freezing. Table 1060:I lists preservation methods by constituent. See Section 7010B for sample collection and preservation requirements for radionuclides.

The foregoing discussion is by no means exhaustive and comprehensive. Clearly, it is impossible to prescribe absolute rules for preventing all possible changes. Additional advice will be found in the discussions under individual determinations, but to a large degree, the dependability of an analytical determination rests on the experience and good judgment of the person

collecting the sample. Numbers of samples required for confidence levels in data quality objectives, however, rely on statistical equations, such as those discussed earlier.

3. Reference

1. WATER POLLUTION CONTROL FEDERATION. 1986. Removal of Hazardous Wastes in Wastewater Facilities—Halogenated Organics; Manual of Practice FD-11. Alexandria, Va.

4. Bibliography

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