Table 1050:6. Examples of Alternative Expressions for Some Analytical Results

Analyte	Units	Possible Reporting Nomenclature
Alkalinity	mg/L	mg/L as $CaCO_3$ or mg/L as HCO_3^-
Ammonia, un-ionized	mg/L	mg/L as N or mg/L as NH_3
Ammonium	mg/L	mg/L as N or mg/L as NH_4^+
Bicarbonate	mg/L	mg/L as HCO_3^- or mg/L as $CaCO_3$
Calcium	mg/L	mg/L as Ca^{2+} or mg/L as $CaCO_3$
Carbonate	mg/L	mg/L as CO_3^{2-} or mg/L as $CaCO_3$
Conductivity	µs/m	µS/m at 25 °C or µS/m at t °C
Hydrogen sulfide	mg/L	mg/L as H_2S or mg/L as S^{2-}
Magnesium	mg/L	mg/L as Mg^{2+} or mg/L as $CaCO_3$
Nitrate	mg/L	mg/L as N or mg/L as NO_3^-
Nitrite	mg/L	mg/L as N or mg/L as NO_2^-
PCB	mg/L	mg/L as PCB or mg/L as decachlorobiphenyl or mg/L as Aroclor mixture
pH	—	pH at 25 °C or pH at t °C
Silicon	mg/L	mg/L as Si or mg/L as SiO_2
Sulfide	mg/L	mg/L as S or mg/L as H_2S
Total dissolved solids	mg/L	mg/L at 180 °C or mg/L at t °C
Uranium	mg/L	mg/L as U or mg/L as U_3O_8
	pCi/L	pCi/L as natural isotopic abundance or pCi/L as specified isotopic abundance
Zinc	mg/L	mg/L Zn as total or mg/L Zn as dissolved
	mg/kg	mg Zn/kg wet or mg Zn/kg dry

Chemical equivalents such as nitrate nitrogen are used to ease accounting of nitrogen chemical forms by allowing them to be added. In the case of organic nitrogen, the analyst may not know the exact organic form of nitrogen analyzed, so for convenience the compound is reported in terms of nitrogen rather than the actual organic compound.

Other concepts such as alkalinity are expressed in terms of calcium carbonate because the true form is not always known. Include other information such as temperature of pH measurement, tempe
measuremen
metals, are d
Although
always be do

Table 1050:6 gives examples of a number of analytes that may be found expressed in other terms. Include complete information on the analyte, because many times the data may be used in other databases. The user may not always be able to assume the chemical form or special physical conditions under which the analyte was analyzed and reported.

3. Propagation of Error

Frequently, an analytical procedure consists of a number of steps in which one measurement is used in computing another measurement. Each measurement has an associated error. The greater the number of measurements used to obtain a computed result, the greater the possible associated error. This process is known as propagation of error.[3] When errors are compounded, they usually increase, but never decrease.

In the propagation of error, we are considering the value of the measurement as well as its associated uncertainty. All measurements have an associated value of uncertainty. The only measurements that have no uncertainty are numbers of events (counts), when properly defined, and mathematical constants.

Further information on propagation of error is available elsewhere,[4–6] and Section 1030 presents information on types and sources of error.

References

1. Texas Instruments. Personal programming. Dallas (TX): Texas Instruments; 1977.
2. Hewlett Packard. Advanced scientific calculator; Owner's Manual, HP-28S. 4th ed. Corvallis (OR): Hewlett Packard Co.; 1988.
3. Harris DC. Quantitative chemical analysis. New York (NY): W.H. Freeman & Co.; 1982.
4. Scarborough JG. Numerical mathematical analysis, 3rd ed. Baltimore (MD): Johns Hopkins Press; 1955.
5. Guedens WJ, Yperman J, Mullens J, VanPoucke LC, Pauwels EJ. Statistical analysis of errors: A practical approach for an

s. J Chem Edu.
LC, Pauwels EJ.
or an undergrad-
s. J Chem Edu.

General Considerations for the Collection & Preservation Water of Samples for Chemical Analysis

1060 COLLECTION AND PRESERVATION OF SAMPLES

Reviewed by Standard Methods Committee, 2011. Editorial revisions, 2021. 2011 revisions by L. Malcom Baker, Rodger B. Baird, Nilda B. Cox, Andrew D. Eaton. Joint Task Group: Lawrence H. Keith (chair), Clifford G. Annis, Gary L. DeKock, Carleton P. Edmunds, Scott J. Mickelson, Mark Wyzalek.

1060 A. INTRODUCTION

The result of any testing method can be no better than the sample on which it is performed. However, it is beyond the scope of *Standard Methods for the Examination of Water and Wastewater* to specify detailed procedures for the collection of all samples because of varied purposes and analytical procedures. Detailed information may be presented in specific methods and that information is to be followed when available. This section presents general considerations for the collection and preservation of

samples applicable primarily to chemical analyses. See appropriate sections for samples to be used in toxicity testing (Section 8020) and microbiological (Sections 9020 and 9060), biological (Part 10 000), and radiological (Section 7010) 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 are the same in the samples as in the material being sampled and that the samples are 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 meet regulatory and 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. Always use sample containers that are clean and free of contaminants. Bake all bottles to be used for organic-analysis sampling at 450 °C.

Fill sample containers that are clean and free of contaminants directly with the sample. Do not prerinse the container with sample because this results in the loss of any pre-added preservative and sometimes can bias results high when certain sample components adhere to the sides of the container. Depending on the 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 the analysis of volatile organic compounds or radon, leave an air space of at least 1% of the container's volume to allow for thermal expansion during shipment, or as may otherwise be required by a method to ensure proper mixing (by inverting or shaking) before sample withdrawal for analysis.

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 when a composite sample is required by a regulatory agency, the composite sample is refrigerated throughout the collection process to limit the instabilities of a compound and its properties.

Sample carefully so that analytical results are as representative as possible of the 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 for each sample location and type. Add this same amount of acid to the samples collected at the location and of the same type. 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 filtered samples are 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 after returning these samples to the laboratory. Often, slight turbidity can be tolerated if experience shows that it causes 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 or not 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 does not fit on a label or attached tag, record the 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, nonsolvent-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 1060 B.2), which trace sample history from collection to final reporting.

Before collecting samples from distribution systems, flush lines with 3 to 5 pipe volumes (or until water is being drawn from the main source) to obtain a representative sample from the supply, taking into account the volume of the 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 3 to 10 well volumes) to ensure that the sample represents the groundwater. Purging stagnant water is critical. Sometimes it is necessary to pump at a specified rate to achieve a characteristic drawdown, if this determines the zones from which the well is supplied. Record the 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 1060 B.1*c*.

Rivers, streams, lakes, and reservoirs are subject to considerable variations from normal causes (e.g., seasonal stratification, diurnal variations, rainfall, runoff, and wind). Choose the 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 the sampling container below the 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, take measures to prevent the loss of sample constituents during compositing. 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 models[1]; verify that the clothing chosen offers 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.

References

1. Forsberg K, Keith LH. Instant Gloves and CPC Database. Austin (TX): Instant Reference Sources, Inc.; 1998.
2. Water Pollution Control Federation. Removal of hazardous wastes in wastewater facilities—halogenated organics; manual of practice FD-11. Alexandria (VA): Water Pollution Control Federation; 1986.

(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 collected from a single selected location, depth, and time. Depth-integrated grab samples are collected over a predetermined area

(integrated for a horizontal spatial representation) or the entire depth of a water column (integrated for a vertical spatial representation), at a selected location and time in a given body of water.

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

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 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 a loss of analyte relationships in individual samples, the potential dilution of analytes below detection levels, increased potential analytical interferences, and an increased possibility of analyte interactions. In addition, the 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 such as 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 to perform, 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

The process of tracing the possession and handling of a sample from the time of collection through analysis and final disposition is referred to as the sample's chain of custody. Properly designed and executed chain-of-custody forms document the sample integrity and that proper handling has occurred from sample collection to data reporting. Sample chain of custody 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 and provide documentation for quality assurances reviews.

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 a seal so that it must be broken to open the sample container or the sample shipping container (e.g., a cooler). Affix the seal to the container before the sample leaves the 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 the process producing the waste stream. Also provide suspected sample composition, including concentrations; number and volume of samples taken; description of sampling point and sampling method; date and time of collection; collector's sample identification numbers; sample distribution and manner of transport; references such as 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 to reconstruct the sampling event without reliance on the collector's memory. Protect the 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 the samples to the 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 the samples to the 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 the sample in the laboratory log book 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. Dispose of samples 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 used increasingly. 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 collected.

c. Sorbent sampling: The 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 (Pyrex

or equivalent) 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 the 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 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 t test 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 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).

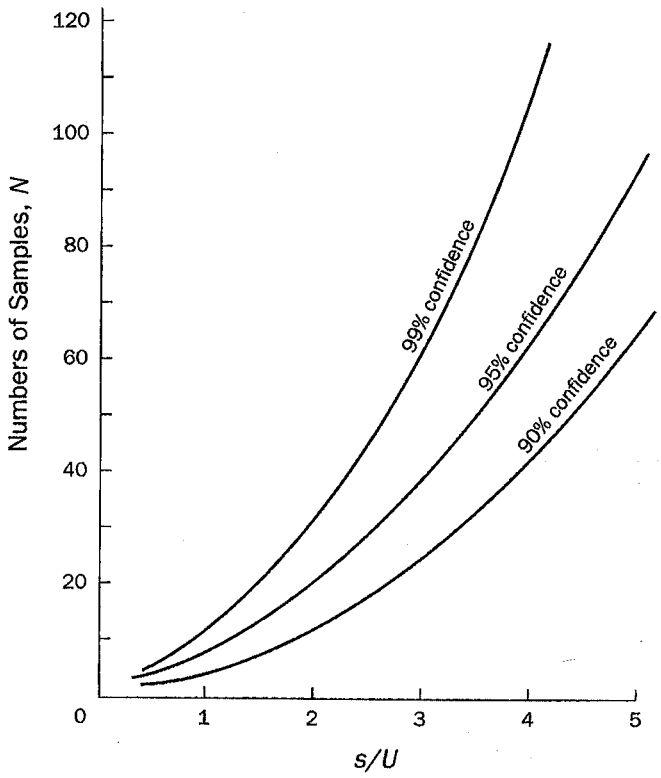

Figure 1060:1. Approximate number of samples required in estimating a mean concentration. Source: Methods for the examination of waters and associated materials: general principles of sampling and accuracy of results. London, England: Her Majesty's Stationery Office, 1980.

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 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 available[7] 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 less than 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 more than 10%, iterative solution of a binomial equation is necessary.[5,8]

6. Sample Volumes

Collect a 1-L sample for most physical and chemical analyses. For certain determinations, larger samples may be necessary. Table 1060:1 lists general guidance for volumes ordinarily required for analyses, but it is strongly recommended that the laboratory staff who 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.

References

1. U.S. Environmental Protection Agency. Test methods for evaluating solid waste: physical/chemical methods, 3rd ed.; Pub. No. SW-846. Washington DC: Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency; 1986.
2. U.S. Environmental Protection Agency. NEIC policies and procedures; EPA-330/9/78/001/-R (rev. 1982). Washington DC: U.S. Environmental Protection Agency, 1982.
3. Water Pollution Control Federation. Removal of hazardous wastes in wastewater facilities—halogenated organics; Manual of Practice FD-11. Alexandria (VA): Water Pollution Control Federation; 1986.
4. Methods for the examination of waters and associated materials: general principles of sampling and accuracy of results. London, England: Her Majesty's Stationery Office; 1980.
5. Keith LH, Patton GL, Lewis DL, Edwards PG. Determining numbers and kinds of analytical samples, Chapter 1. In: Principles of environmental sampling, 2nd ed. Washington DC: American Chemical Society; 1996 (ACS Professional Reference Book).
6. Keith LH. Compilation of EPA's sampling and analysis methods, 2nd ed. Boca Raton (FL): CRC Press; 1996.
7. Gilbert RO. Statistical methods for environmental pollution monitoring. New York (NY): Van Nostrand Reinhold, 1987.
8. Grant EL, Leavenworth RS. Statistical quality control, 6th ed. New York (NY): McGraw-Hill, Inc.; 1988.

(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 below 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 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 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

Table 1060:1. Summary of Special Sampling and Handling Requirements[a]

Determination	Container	Minimum Sample Size (mL)	Sample Type	Preservation[b]	Maximum Storage Recommended	Regulatory[c]
Acidity	P, G(B), FP	100	g	Cool to ≤6°C	24 h	14 d
Alkalinity	P, G, FP	200	g	Cool to ≤6°C	24 h	14 d
BOD	P, G, FP	1000	g, c	Cool to ≤6°C	6 h	48 h
Boron	FP (PTFE), G (quartz)	1000	g, c	HNO_3 to pH<2	28 d	6 months
Bromide	P, G, FP	100	g, c	None required	28 d	28 d
Carbon (organic and total)	G(B), P, FP	100	g, c	Analyze immediately or cool to ≤6°C; and add HCl, H_3PO_4, or H_2SO_4 to pH<2	7 d	28 d
Carbon dioxide	P, G	100	g	Analyze immediately	15 min	N.S.
COD	P, G, FP	100	g, c	Analyze as soon as possible, or add H_2SO_4 to pH<2; Cool to ≤6 °C	7 d	28 d
Chloride	P, G, FP	50	g, c	None required	N.S.	28 d
Chlorine (total and residual)	P, G	500	g	Analyze immediately	15 min	0.25 h
Chlorine dioxide	P, G	500	g	Analyze immediately	15 min	N.S.
Chlorophyll	P, G (amber)	500	g	Unfiltered, dark, ≤6 °C. Filtered, dark, –20 °C (Do not store in frost-free freezer)	48 h; 28 d if algae removed from water having pH > 6 onto glass fiber filter, placed in airtight plastic bags and frozen	N.S.
Color	P, G, FP	500	g, c	Cool to ≤6 °C	24 h	48 h
Specific conductance	P, G, FP	500	g, c	Cool to ≤6 °C	28 d	28 d
Cyanide						
Total	P, G, FP	100	g	Add NaOH to pH 12 for SDWA compliance and pH 11 for all other samples. If sample is to be stored, cool to ≤6 °C in dark. Remove interferences according to method specific instructions	48 h	14 d; 24 h if >50 mg/L sulfide present
Amenable to chlorination	P, G, FP	500	g	Remove interferences according to method specific instructions; add NaOH to pH 11 and cool to ≤6 °C	48 h	14 d; 24 h if >50 mg/L sulfide present
Free	P, G, FP	100	g	Remove interferences according to method specific instructions; add NaOH to pH 11 and cool to ≤6 °C	48 h	14 d
Weak and dissociable (WAD)	P, G, FP	100	g	Add NaOH to pH 12 for SDWA compliance and pH 11 for all other samples. If sample is to be stored, cool to ≤6 °C in dark. Remove interferences according to method specific instructions	48 h	14 d; 24 h if >50 mg/L 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 pH <2	6 months	6 months
Hydrogen peroxide	P, G	50	g	Analyze immediately	10 min after collection	
Iodine	P, G	500	g	Analyze immediately	15 min.	N.S.
Metals	P(A), G(A), FP(A)	1000	g, c	For dissolved metals filter immediately; add HNO_3 to pH <2	6 months	6 months
Chromium VI	P(A), G(A), FP(A)	250	g	Cool to ≤6 °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	–[a]	–	g, c	–	–	
Ferrous iron	P(A), G(A), FP(A)	500	g	Add 2 ml conc HCL to aliquot. Add 20 mL phenanthroline solution and 10 mL $NH_2C_2H_3O_2$ to 50 mL portion of acidified sample. Analyze immediately		15 min

Table 1060:1. Continued

Determination	Container	Minimum Sample Size (mL)	Smple Type	Preservation[b]	Maximum Storage Recommended	Regulatory[c]
Mercury	P(A), G(A), FP(A)	500	g, c	Add HNO_3 to pH <2, Cool ≤6 °C	28 d	28 d
Nitrogen						
Ammonia	P, G, FP	500	g, c	Analyze as soon as possible or add H_2SO_4 to pH <2. Cool to 6 °C	7 d	28 d
Nitrate	P, G, FP	100	g, c	Analyze as soon as possible; cool to ≤6 °C	48 h	48 h (14 d for chlorinated samples)
Nitrate + nitrite	P, G, FP	200	g, c	Add H_2SO_4 to pH <2; cool to ≤6 °C	48 h	28 d
Nitrite	P, G, FP	100	g, c	Analyze as soon as possible; cool to ≤6 °C	none	48 h
Organic, Kjeldahl	P, G, FP	500	g, c	Cool to ≤6 °C. Add H_2SO_4 to pH <2	7 d	28 d
Total	P, G. FP	100	g, c	Cool to ≤6 °C, add H_2SO_4, or HCl to pH ≤2	7 d	28 d
Odor	G	500	g	Analyze as soon as possible; cool ≤6 °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 pH <2, Cool to ≤6 °C	28 d	28 d
Organic Compounds						
MBAS	P, G, FP	250	g, c	Cool to ≤6 °C	48 h	48 h
Pesticides[a]	G(S, PTFE-lined cap)	1000	g, c	Cool to ≤6 °C. Add 1000 mg/L ascorbic acid if residual chlorine present (0.008% sodium thiosulfate in CFR 136)	7 d	7 d until extraction; 40 d after extraction
Phenols (5530)	G	500	g	Cool to ≤6 °C. Add H_2SO_4 to pH <2	See footnote[a]	28 d until extraction, 2 d after extraction
Phenols (6420)	G (PTFE-lined cap)	1000	g, c	Cool to ≤6 °C. add 0.008% sodium thiosulfate if chlorine is present	7 d	7 d until extraction; 40 d after extraction
Purgeables[a] (purge and trap)	G (PTFE-lined cap)	2 × 40	g	Cool, ≤6 °C; add HCl to pH <2; add 1000 mg/L ascorbic acid if residual chlorine present (0.008% sodium thiosulfate in CFR 136)	7 d	14 d
Base/neutrals and acids	G(S, amber)	1000	g, c	Cool to ≤6 °C; add 0.008% sodium thiosulfate in CFR 136 if chlorine is present	7 d	7 d until extraction; 40 d after extraction
Oxygen (dissolved)	G-, P-BOD bottle	300	g			
Electrode				Analyze immediately	15 min	15 min
Winkler				Titration may be delayed after acidification	8 h	8 h
Ozone	G	1000	g	Analyze immediately	15 min	N.S.
Peracetic acid	G, P	50	g	Analyze immediately	10 min after collection	
pH	P, G	50	g	Analyze immediately	15 min	15 min
Phosphate	G(A)	100	g	For dissolved phosphate filter immediately. Cool to ≤6 °C	48 h	48 h
Phosphorus, total	P, G, FP	100	g, c	Add H_2SO_4 to pH <2 and cool to ≤6 °C	28 d	28 d
Salinity	G (wax seal)	240	g	Analyze immediately or use wax seal	6 months	N.S.
Silica	FP (PTFE), G (quartz)	200	g, c	Cool to ≤6 °C; do not freeze	28 d	28 d

Table 1060:1. Continued

Determination	Container	Minimum Sample Size (mL)	Smple Type	Preservation[b]	Maximum Storage Recommended	Regulatory[c]
Sludge digester gas	G, gas bottle	—	g	—	N.S.	
Solids[1]	P, G	200	g, c	Cool to ≤6 °C	7 d	2–7 d; see reference 1
Sulfate	P, G, FP	100	g, c	Cool to ≤6 °C	28 d	28 d
Sulfide	P, G, FP	100	g, c	Cool to ≤6 °C. Add 4 drops of 2 N zinc acetate per 100 mL. Add NaOH to pH >9	48 h	7d
Temperature	P, G, FP	—	g	Analyze immediately	15 min	0.25 h
Turbidity	P, G, FP	100	g, c	Analyze same day. Store in dark up to 24 h. Cool to ≤6 °C	24 h	48 h

Abbreviations: c = composite; g = grab; G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO_3; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents or baked; FP = fluoropolymer (PTFE); N.S. = not stated in cited reference; P = plastic (polyethylene or equivalent).

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

[b] 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.

[c] See reference 2 for possible differences regarding container and preservation requirements.

Note 1: 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 minutes without demonstrating that cyanide (CN) is stable for longer periods in a specific matrix.

Note 2: 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.

References

1. U.S. Environmental Protection Agency. 2007. 40 CFR Part 136, Table II.

2. U.S. Environmental Protection Agency. 1992. Rules and Regulations. 40 CFR Parts 100–149.

nitrogen and phosphorus cycles are examples of biological influences on sample composition.

Zero headspace is important in the preservation of samples with volatile organic compounds and radon. Avoid a loss of volatile materials by collecting samples in a completely filled container. Achieve this by carefully filling a bottle so the top of the 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 the 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 the bottle with the 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 the collection of a sample and its analysis, the more reliable are 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 1060:1). 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 1060:1. Record the 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 1060:1 lists preservation methods by constituent. See Section 7010 B 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.

Reference

1. Water Pollution Control Federation. Removal of hazardous wastes in wastewater facilities—halogenated organics; Manual of Practice FD-11. Alexandria (VA): Water Pollution Control Federation; 1986.

Bibliography

Keith LH. Principles of environmental sampling, 2nd ed. Washington DC: American Chemical Society; 1996 (ACS Professional Reference Book).

1080 REAGENT WATER

Reviewed by Standard Methods Committee, 2011. Editorial revisions, 2021.

1080 A. INTRODUCTION

One of the most important aspects of analysis is preparing the reagent water used for blanks and reagents. *Reagent water* is water with no detectable concentration of the compound or element to be analyzed (i.e., it is below the analytical method's detection level). Reagent water should also be free of substances that interfere with analytical methods. However, its overall quality (concentrations of organic, inorganic, and biological constituents) depends on the water's intended uses.

Use any method to prepare reagent water that meets the applicable quality requirements. Various combinations of reverse osmosis, distillation, deionization, or ultrafiltration and ultraviolet irradiation can produce reagent water. Keep in mind, however, that improperly operated or maintained water purification systems may add rather than remove contaminants.

This section provides general guidelines for preparing reagent water. Table 1080:1 lists commonly available water purification processes and the major classes of contaminants that they remove. For details on preparing water for microbiological tests, see Section 9020 B.4*d*.

1080 B. METHODS FOR PREPARING REAGENT-GRADE WATER

1. Distillation

Distillation is the process of heating a liquid until it boils, capturing and cooling the resultant hot vapors, and collecting the condensed vapors. Laboratory-grade distilled water should be generated in a still made of all-borosilicate glass, fused quartz, tin, or titanium. To remove ammonia, distill from an acid solution. Remove CO_2 by boiling the water for 15 min and cooling rapidly to room temperature; exclude atmospheric CO_2 by using a tube containing soda lime or a commercially available CO_2-removing agent (e.g., Ascarite II).

Impurities may leach into water from its container during boiling. Also, freshly replaced filters, cartridges, and resins initially can release impurities. Pretreat feedwater and maintain a still periodically to minimize scale formation. A demineralization pretreatment using reverse osmosis or ion exchange may be required if the feedwater contains significant concentrations of calcium, magnesium, and bicarbonate ions.

2. Reverse Osmosis

During reverse osmosis, water is forced under pressure through a semipermeable membrane, thereby removing some dissolved constituents and suspended impurities. The resultant reagent water quality depends on both feedwater quality and the type and condition of membranes used.