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Water Analysis Guide

09/2013, Edition 1

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Acid/Base										•			•	•				•	•						
Acidity		•				•			•	•	•		•					•	•		•				•
Alkalinity	•	•	•	•	•	•			•	•	•	•			•	•					•	•		•	•
Aluminum			•		•	•			•									•	•	•			•	•	
Arsenic									•	•	•						•			•			•	•	•
Ascorbic Acid				•																					
Bacteria		•		•	•				•	•	•	•		•	•	•	•	•	•	•		•	•	•	•
Barium						•					•			•	•								•		
BOD				•					•	•	•	•		•	•			•		•	•		•	•	
Boron					•	•				•	•			•	•		•					•	•		•
Bromine				•	•				•		•					•						•		•	
Cadmium						•			•				•						•	•			•	•	
Calcium	•			•	•						•				•							•			•
Carbon Dioxide		•		•	•				•	•	•														•
Chelants					•	•			•								•								•
Chloride	•	•		•	•	•	•		•	•	•	•	•	•	•		•		•		•	•	•	•	•
Chlorine	•	•	•	•	•		•	•	•	•	•	•		•	•	•	•	•			•	•	•	•	•
Chlorine Dioxide				•	•				•			•				•		•			•				
Chromate													•						•						
Chromium (Hexavalent)					•	•			•	•	•		•	•									•	•	
Chromium (Total)					•	•				•	•		•	•						•	•		•	•	•
Cobalt						•				•	•		•	•									•	•	
COD				•		•	•	•	•	•	•	•		•	•		•	•		•	•		•	•	
Color				•					•	•	•				•			•			•		•	•	
Conductivity	•	•		•	•	•			•	•	•			•			•					•	•	•	
Copper		•	•	•	•	•			•	•	•		•			•	•		•	•	•	•	•	•	•
Cyanide									•		•		•		•								•	•	
Cyanuric Acid						•			•		•					•									
Detergents						•				•	•													•	•
Dissolved Oxygen	•	•		•	•			•	•	•	•	•						•			•	•	•	•	•
Erythorbic Acid					•	•								•			•								•
Fluoride				•					•										•			•	•		•
Formaldehyde						•					•										•				•

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Gluteraldehyde						•			•	•														•	
Glycols						•			•		•			•			•								•
Hardness	•	•	•	•	•	•		•	•	•	•	•		•	•	•	•	•			•	•	•	•	•
Hydrazine					•												•								
Hydrogen Peroxide						•			•	•	•		•		•				•			•			•
Hydrogen Sulfide						•			•					•										•	•
Iodide						•																			
Iodine			•	•		•			•		•	•			•									•	
Iron (Ferrous)					•	•			•				•												•
Iron (Total)	•		•	•	•	•		•	•	•	•	•		•			•				•	•	•	•	•
Lead			•						•	•	•		•				•		•	•			•	•	•
Manganese				•			•		•														•		•
Mercury							•		•											•			•	•	•
Molybdenum				•	•	•	•				•				•		•			•	•		•		•
Nickel						•			•		•		•							•			•	•	•
Nitrogen Ammonia	•	•	•		•	•			•	•	•	•		•			•				•	•		•	•
Nitrogen (Inorganic)						•				•	•													•	
Nitrogen (Total)						•			•	•	•										•		•	•	
Nitrogen (Nitrate)	•	•	•	•	•				•	•	•	•									•	•	•	•	•
Nitrogen (Monochloramine)									•														•	•	
Nitrogen (TKN)	•					•			•	•	•	•								•			•	•	
Nitrogen (Nitrite)		•	•		•				•	•	•													•	
Oil and Grease						•						•	•	•						•			•		
Oxygen Scavenger					•	•								•			•								•
Ozone		•		•	•			•	•		•				•							•		•	•
PCB											•						•			•		•	•	•	
Permanganate									•								•								
pH	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Phenols						•				•	•			•			•				•		•		•
Phosphate	•	•	•	•	•	•	•		•	•		•					•	•		•	•	•	•	•	•

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Phosphonates					•	•			•	•	•													•	•
Phosphorus	•					•	•		•	•	•	•		•			•				•		•	•	•
Potassium	•								•		•			•								•			
QAC					•	•					•							•							•
Salinity	•	•				•			•	•	•			•						•					•
Selenium									•	•	•				•					•			•		•
Silica	•			•	•	•	•		•	•	•	•			•		•	•	•		•	•			•
Silver						•			•	•	•		•	•	•	•	•	•	•	•			•	•	•
Sodium	•			•					•								•	•	•			•	•	•	•
Sodium Chromate									•	•	•						•								
Sodium Hydroxide													•						•						
Sulfate	•					•			•	•	•						•				•	•	•		•
Sulfide						•			•		•			•									•	•	•
Sulfite					•	•			•	•	•						•	•					•	•	•
Tannin					•				•		•														•
TDS		•		•	•				•	•													•		•
Toxicity						•			•		•									•			•	•	
TPH						•			•		•						•						•		
Triazole					•	•											•								
Turbidity		•		•	•				•	•	•	•	•		•		•	•	•	•		•	•	•	•
Volatile Acids									•	•	•			•										•	
Water in Oil											•		•	•											
Zinc					•	•			•	•	•		•					•		•	•		•	•	•

Section 2 Abbreviations and conversions

2.1 Procedure abbreviations

Table 1 shows common abbreviations used in written chemical procedures.

Table 1 Abbreviations

Abbreviation	Definition		Abbreviation	Definition
°C	degree(s) Celsius (Centigrade)		L	liter—volume equal to one cubic decimeter (dm^3)
°F	degree(s) Fahrenheit		LR	low range
ACS	American Chemical Society reagent grade purity		MDL	method detection limit
APHA Standard Methods	Standard Methods for the Examination of Water and Wastewater, published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation (WEF), is the standard reference work for water analysis. Many procedures contained in this manual are based on Standard Methods.		MDB	marked dropper bottle
			mg/L	milligrams per liter (ppm)
			µg/L	micrograms per liter (ppb)
			mL	milliliter—1/1000 of a liter. It is approximately the same as a cubic centimeter (and is sometimes called a “cc”).
			MR	medium range
			NIPDWR	National Interim Primary Drinking Water Regulations
AV	AccuVac®		NPDES	National Pollutant Discharge Elimination System
Bicn	bicinchoninate		P	phosphorus
conc	concentrated		PCB	poly chlorinated biphenyl
DB	dropping bottle		ppb	parts per billion
DBP	disinfection by-products		ppm	parts per million
CFR	Code of Federal Regulations		RL	Rapid Liquid™
EDL	Estimated detection limit		SCDB	self-contained dropping bottle
EPA	Environmental Protection Agency		THM	trihalomethane
F&T	free and total		TNT	Test ‘N Tube™
FM	FerroMo®		TOC	total organic carbon
FV	FerroVer®		TPH	total petroleum hydrocarbons
FZ	FerroZine®		TPTZ	2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine
g	grams		USEPA	United States Environmental Protection Agency
gr/gal	grains per gallon (1 gr/gal = 17.12 mg/L)		ULR	ultra low range
HR	high range			

2.2 Conversions

2.2.1 Chemical species

Table 2 shows species conversion factors for many commonly used chemicals.

Table 2 Conversion factors

To convert from...	To...	Multiply by...
mg/L Al	mg/L Al_2O_3	1.8895
mg/L B	mg/L H_3BO_3	5.7
mg/L Ca-$CaCO_3$	mg/L Ca^{2+}	0.4004
mg/L $CaCO_3$	mg/L Ca^{2+}	0.4004
mg/L $CaCO_3$	mg/L Mg^{2+}	0.2428
µg/L Carbohydrazide	µg/L Hydroquinone	1.92
µg/L Carbohydrazide	µg/L ISA	2.69
µg/L Carbohydrazide	µg/L MEKO	3.15
mg/L Cr^{6+}	mg/L CrO_4^{2-}	2.231
mg/L Cr^{6+}	mg/L Na_2CrO_4	3.115
mg/L Cr^{6+}	mg/L $Cr_2O_7^{2-}$	2.077
mg/L Mg-$CaCO_3$	mg/L Mg^{2+}	0.2428
mg/L Mn	mg/L $KMnO_4$	2.876
mg/L Mn	mg/L MnO_4^-	2.165
mg/L Mo^{6+}	mg/L MoO_4^{2-}	1.667
mg/L Mo^{6+}	mg/L Na_2MoO_4	2.146
mg/L N	mg/L NH_3	1.216
mg/L N	mg/L NO_3^-	4.427
mg/L Cl_2	mg/L NH_2Cl	0.726
mg/L Cl_2	mg/L N	0.197
mg/L NH_3-N	mg/L NH_3	1.216
mg/L NH_3-N	mg/L NH_4^+	1.288
mg/L NO_2^-	mg/L $NaNO_2$	1.5
mg/L NO_2^-	mg/L NO_2^-–N	0.3045
mg/L NO_2^--N	mg/L $NaNO_2$	4.926
µg/L NO_2^--N	µg/L $NaNO_2$	4.926
mg/L NO_2^--N	mg/L NO_2^-	3.284
µg/L NO_2^--N	µg/L NO_2^-	3.284
mg/L NO_3^--N	mg/L NO_3^-	4.427
mg/L PO_4^{3-}	mg/L P	0.3261
µg/L PO_4^{3-}	µg/L P	0.3261
mg/L PO_4^{3-}	mg/L P_2O_5	0.7473
µg/L PO_4^{3-}	µg/L P_2O_5	0.7473

Table 2 Conversion factors (continued)

To convert from...	To...	Multiply by...
mg/L SiO_2	mg/L Si	0.4674
µg/L SiO_2	µg/L Si	0.4674

2.2.2 Hardness conversion

Table 3 shows the factors to convert hardness from one unit of measure to another. For example, to convert mg/L $CaCO_3$ to German parts/100,000 CaO, multiply the value in mg/L x 0.056.

Note: *meq/L = N × 1000*

Table 3 Hardness conversion factors

Units of measure	mg/L $CaCO_3$	British gr/gal (Imperial) $CaCO_3$	American gr/gal (US) $CaCO_3$	French parts/ 100,000 $CaCO_3$	German parts/ 100,000 $CaCO_3$	meq/L [1]	g/L CaO	lb/ft^3 $CaCO_3$
mg/L $CaCO_3$	1.0	0.07	0.058	0.1	0.056	0.02	$5.6x10^{-4}$	$6.23x10^{-5}$
English gr/gal $CaCO_3$	14.3	1.0	0.83	1.43	0.83	0.286	$8.0x10^{-3}$	$8.9x10^{-4}$
US gr/gal $CaCO_3$	17.1	1.2	1.0	1.72	0.96	0.343	$9.66x10^{-3}$	$1.07x10^{-3}$
French p/100,000 $CaCO_3$	10.0	0.7	0.58	1.0	0.56	0.2	$5.6x10^{-3}$	$6.23x10^{-4}$
German p/100,000 CaO	17.9	1.25	1.04	1.79	1.0	0.358	$1x10^{-2}$	$1.12x10^{-3}$
meq/L	50.0	3.5	2.9	5.0	2.8	1.0	$2.8x10^{-2}$	$3.11x10^{-2}$
g/L CaO	1790.0	125.0	104.2	179.0	100.0	35.8	1.0	0.112
lb/ft^3 $CaCO_3$	16,100.0	1123.0	935.0	1610.0	900.0	321.0	9.0	1.0

[1] epm/L or mval/L

Section 3 Laboratory practices

3.1 Temperature

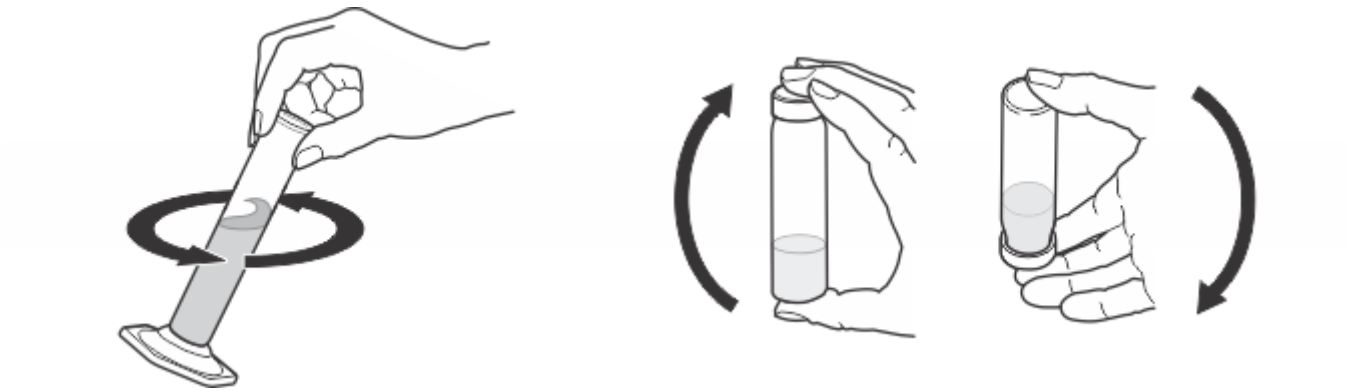
Most methods are completed accurately when the sample temperature is between 20 and 25 °C (68 to 77 °F). A note in the individual procedure shows any special temperature requirements.

3.2 Mixing

When reagent is added to a graduated cylinder or titration flask, swirl the sample gently. A gentle swirl motion decreases the risk of atmospheric contamination in carbon dioxide and other tests for gases.

1. Hold the cylinder (or flask) firmly with the tips of the thumb and first two fingers (Figure 1).
2. Hold the cylinder at a 45-degree angle and make a circular motion from the wrist.
3. Move the cylinder in approximately 305-mm (12-in.) circles. Make enough rotation to complete the mixing in a few turns.

Figure 1 Swirl a cylinder and invert a sample cell



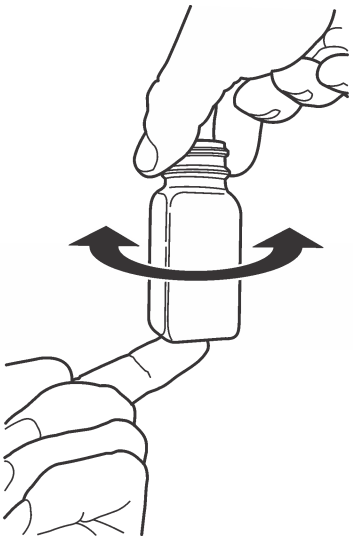
To mix a sample in a closed sample cell or a mixing cylinder:

1. Hold the cell or cylinder, in a vertical position with the cap on top.
2. Invert so that the cap is on the bottom. Return the cell to its original position (Figure 1). Repeat as needed.

To mix a sample in a square sample cell:

1. Hold the neck of the cell with the thumb and index finger of one hand. Put the concave bottom of the cell on the tip of the index finger of the other hand.
2. Rotate the cell quickly one way and then in the reverse direction to mix (Figure 2).

Figure 2 Rotate a sample cell



3.3 Digestion

Several procedures use sample digestion. Digestion uses chemicals and heat to break down a substance into components that can be analyzed. This section briefly describes three different digestion procedures.

The Digesdahl system gives a digested substance applicable for the determination of metals, total phosphorus and total Kjeldahl nitrogen (TKN). It is fast and is very effective at destroying interfering organic materials.

For USEPA reporting purposes, USEPA-approved digestions are necessary. USEPA presents two digestions (mild and vigorous) for metals analysis. Other digestion procedures are necessary for mercury, arsenic, phosphorus and TKN.

Refer to Sample pretreatment by digestion on page 41 for more information on sample digestion.

3.4 Distillation

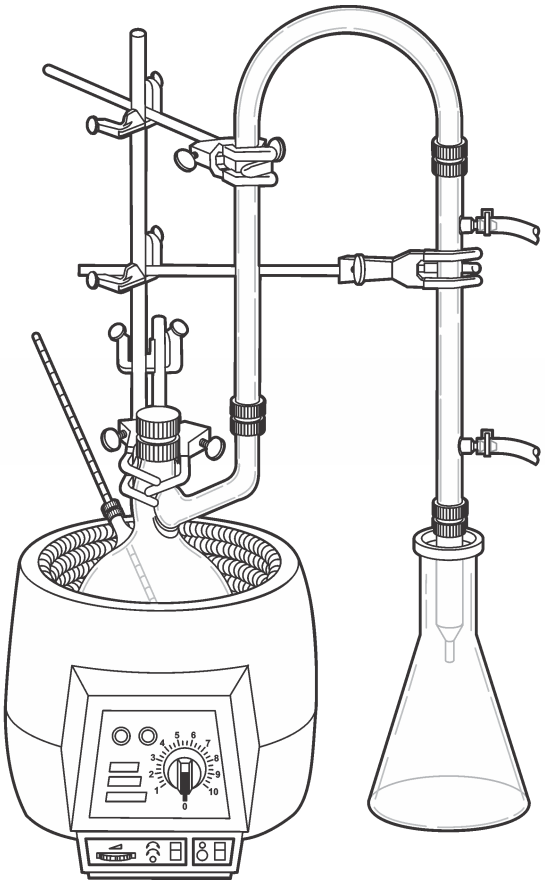
Distillation is an effective and safe method used to separate some chemical components for analysis. The equipment that follows is recommended for distillation:

- General Purpose Distillation Apparatus (22653-00), shown in Figure 3
- Arsenic Distillation Apparatus Set (22654-00)
- Cyanide Distillation Apparatus Set (22658-00)
- General Purpose Heater and Support Apparatus (22744-00, 115 VAC, 60 Hz)
- General Purpose Heater and Support Apparatus (22744-02, 230 VAC, 50 Hz)

The Distillation Apparatus is applicable for water and wastewater that use sample pretreatment by distillation. Applications for the General Purpose Apparatus include: fluoride, albuminoid nitrogen, ammonia nitrogen, phenols, selenium and volatile acids.

The General Purpose Heater and Support Apparatus gives efficient heating and anchoring of the glassware.

Figure 3 General purpose distillation apparatus



3.5 Filtration

Filtration separates particulates from an aqueous sample. Filtration uses a porous medium that keeps particulates but lets liquids pass through. Filtration removes turbidity from water samples. Turbidity can interfere in colorimetric analyses.

The two filtration methods most frequently used are vacuum and gravity filtration.

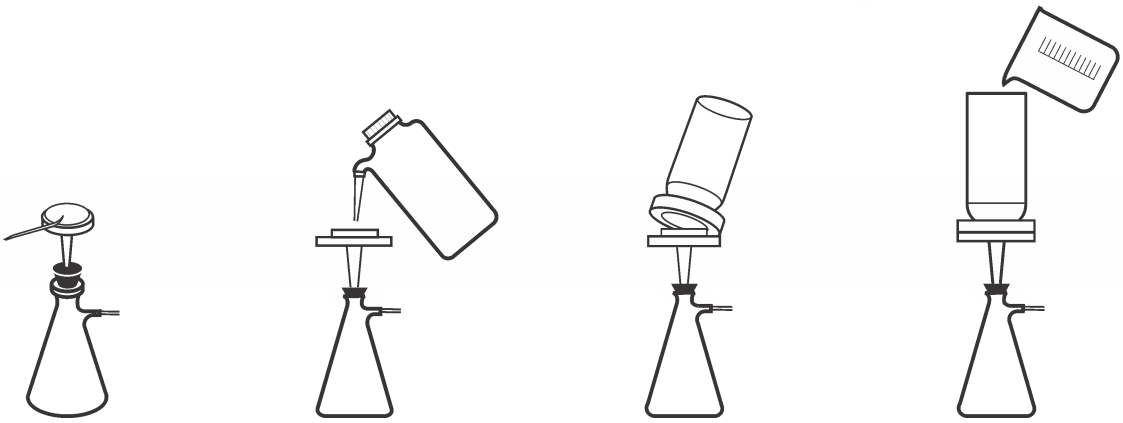
3.5.1 Vacuum filtration

Vacuum filtration uses both suction and gravity to pull the liquid through the filter. An aspirator or vacuum pump is used to make suction (Figure 4). Vacuum filtration is faster than gravity filtration alone.

To filter with a vacuum:

1. Use tweezers to put a filter paper into the filter holder.
2. Put the filter holder assembly in the filtering flask.
3. Dampen a filter paper with deionized water to make sure that there is adhesion to the holder.
4. Put the funnel housing on the filter holder assembly.
5. While a vacuum is applied to the filtering flask, transfer the sample to the filtering apparatus.
6. When the filtration is complete, slowly release the vacuum from the filtering flask and transfer the solution from the filter flask to another container.

Figure 4 Vacuum filtration



3.5.2 Necessary apparatus for vacuum filtration

Description	Unit	Item no.
Filter discs, glass fiber, 47-mm	100/pkg	253000
Filter holder, membrane, 47-mm	each	1352900
Flask, filtering, 500-mL	each	54649
Select one of the following:		
Pump, vacuum, hand operated	each	1428300
Pump, vacuum, portable, 115 VAC	each	2824800
Pump, vacuum, portable, 230 VAC	each	2824801
Tubing, vacuum	—	2074145
Tweezers	each	1428200

3.5.3 Gravity filtration

Many chemical procedures use gravity filtration with the items in Table 4. Gravity filtration is better for fine particles (Figure 5). The rate of filtration increases as the volume increases in the filter cone, but do not fill the cone more than three-quarters full.
Note: *Pretreatment with acid and heat is often necessary for metal tests. Because filter paper does not withstand acid and heat pretreatment, use a glass filter disc in the vacuum filtration. Glass filter discs do not keep color species like the paper filters.*

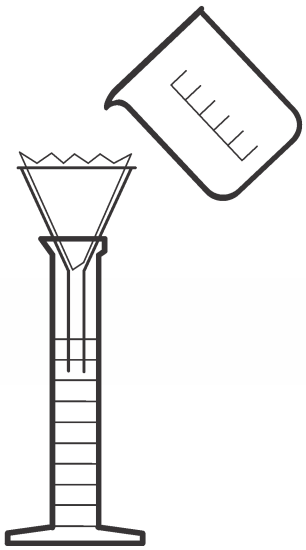
To filter with gravity:

1. Put a folded filter paper into the funnel.
2. Dampen the filter paper with deionized water so that it bonds to the funnel.
3. Put the funnel into an Erlenmeyer flask or graduated cylinder.
4. Pour the sample into the funnel.

Table 4 Necessary apparatus for gravity filtration

Description	Unit	Item no.
Cylinder, graduated, 100-mL	each	50842
Funnel, poly, 65-mm	each	108367
Filter paper, 12.5-cm, pleated	100/pkg	189457
Flask, Erlenmeyer, 125-mL	each	50543

Figure 5 Gravity filtration



3.6 Reagents

3.6.1 Reagent and standard stability

In general, reagents and standards have the maximum shelf life when they are put in a location that is cool, dark and dry. The product label gives any special storage needs.

It is always good laboratory practice to put the date on chemicals upon receipt and to move supplies so that the older supplies are used first. When the reagent shelf life is unknown or in doubt, use a standard to measure reagent effectiveness.

Absorption of moisture, carbon dioxide or other gases from the atmosphere, bacterial action, high temperatures or light (with photosensitive compounds) may affect the reagent shelf life. In some cases, reaction with the storage container or interaction of reagent components may occur.

3.6.2 Reagent blank

In several tests, the contribution of the reagent(s) to the final reading is of such a magnitude that it must be compensated for whenever the test is completed. Reagent blank refers to that portion of the test result contributed solely by the reagent. This makes a positive error in the test results.

Reagents are made with the lowest possible blank. For most reagents, it is less than 0.009 absorbance units. However, it is sometimes impossible or impractical to make reagents with such a low blank. When such reagents are used, it is best to find the reagent blank with the procedure that uses high-quality water (deionized, distilled, etc.) in place of sample to “zero” the instrument. The resulting value is then shown in the concentration units of the test and is subtracted from each sample determination that uses the same reagent lot. Spectrophotometer and colorimeter software lets the reagent blank value be kept and subtracted automatically from each sample value. The reagent blank needs to be found only at first use, when a new lot of reagent has been opened or if contamination is suspected.

In most tests, the reagent blank is so small the instrument may be set to zero on either an untreated portion of the original water sample or on deionized water. This will not result in a significant loss of accuracy unless the test is for very low levels of the species of interest. When a test is for very low levels of the species, it is best to use a reagent blank prepared as above.

3.7 Sample dilution

Most colorimetric tests use volumes of 10 and 25 mL. However, in some tests, the color developed in the sample may be too intense to be measured due to high levels of analyte or unexpected colors may develop due to an interference. In one or the other case, dilute the sample to make a measurable endpoint or to find out if interfering substances are present.

To dilute the sample:

1. Use a pipet to add the selected sample portion to a clean graduated cylinder (or volumetric flask for more accurate work).
2. Fill the cylinder (or flask) to the necessary volume with deionized water.
3. Mix well. Use the diluted sample to complete the test.
   Table 5 shows the relative quantities and multiplication factors to use with a 25-mL graduated cylinder. The concentration of the sample is equal to the diluted sample result multiplied by the multiplication factor.
   Note: *For sample sizes of 10 mL or less, use a pipet to measure and add the sample to the graduated cylinder or volumetric flask.*

Table 5 Sample dilution volumes

Sample volume (mL)	mL of deionized water used to bring the volume to 25 mL	Multiplication factor
25.0	0.0	1
12.5	12.5	2
10.0	15.0	2.5
5.0	20.0	5
2.5	22.5	10
1.0	24.0	25
0.250	24.75	100

More accurate dilutions can be made with a pipet and a 100-mL volumetric flask (Table 6).

1. Use a pipet to add the sample. Dilute to volume with deionized water.
2. Put in the stopper and invert to mix.

Table 6 Multiplication factors for dilution to 100 mL

Sample volume (mL)	Multiplication factor
1	100
2	50
5	20
10	10
25	4
50	2

3.7.1 Sample dilution with interfering substances

Sample dilution may affect the level at which a substance interferes. The effect of the interferences decreases as the dilution increases. In other words, higher levels of an interfering substance can be tolerated in the original sample if it is diluted before analysis.

Example:

Copper does not interfere at or below 100 mg/L for a 25-mL sample in a procedure. If the sample volume is diluted with an equal volume of water, what is the level at which copper will not interfere?

Total volume ÷ Sample volume = Dilution factor

25 ÷ 12.5 = 2

Interference level × Dilution factor = Interference level in sample

100 × 2 = 200

The level at which copper will not interfere in the diluted sample is at or below 200 mg/L.

3.8 AccuVac® Ampuls

⚠CAUTION

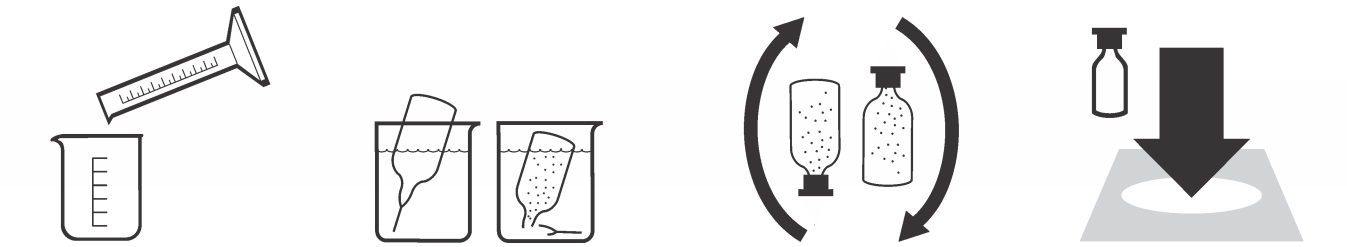
Personal injury hazard. Glass ampules have sharp edges after they are opened. Use personal protective equipment to work with glass ampules.

AccuVac Ampuls contain pre-measured powder or liquid vacuum-packed in optical-quality glass ampules.

To use AccuVac Ampuls:

1. Collect the sample in a beaker or other open container.
2. Use one of the methods that follow to break the tip off the ampule:
   - Use the optional AccuVac Snapper (2405200). Refer to Use the AccuVac Snapper on page 19 for instructions.
   - Put the ampule tip well below the sample surface and break the tip off against the beaker wall (Figure 6). The break must be far enough below the surface that air does not come in as the level of the sample drops.
3. Secure an ampule cap over the tip of the ampule. Invert the ampule several times to dissolve the reagent. The cap protects from broken glass and supplies a grip to insert and remove the ampul from the cell holder. Wipe the ampule with a lint-free cloth to remove fingerprints.
   Note: *Without the cap, the liquid stays in the ampule when the ampule is inverted.*
4. Insert the ampule into the sample cell holder and read the results directly.

Figure 6 Use the AccuVac Ampuls

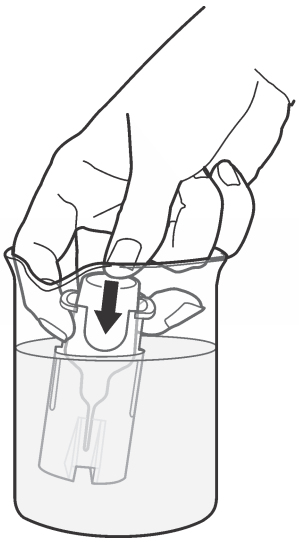


3.8.1 Use the AccuVac Snapper

1. Hold the snapper with the open end up.
2. Gently slip the ampule into the snapper, point first, until the tip touches the ramp at the bottom of the snapper.
3. Hold the snapper between the index and middle finger (like a syringe). With the ampule tip down, lower the snapper into the sample until the ampule shoulder is wet.
4. Push on the flat end of ampule with the thumb (as if depressing the plunger on a syringe) until the tip snaps (Figure 7). Let the ampule fill before the sample is removed.

5. Rinse the wet end of the snapper and ampule with clean water, if necessary. Remove the ampule from the snapper.
6. Discard the ampule tip (kept in the snapper) in an applicable waste receptacle.

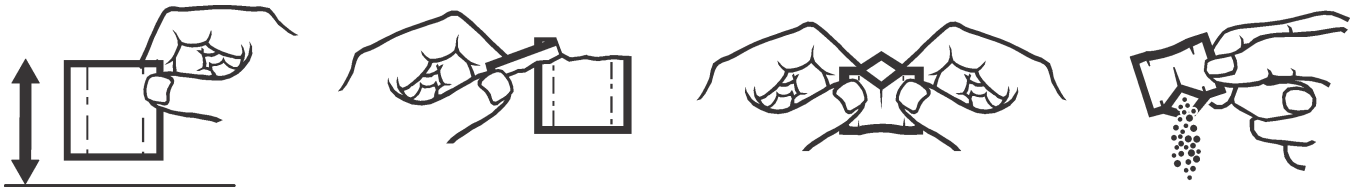
Figure 7 Use the AccuVac Snapper



3.9 PermaChem® pillows

PermaChem pillows use powdered reagents to minimize deterioration and the risk of reagent spills (Figure 8). Hold the pillow away from the face as it is opened.

Figure 8 Open the PermaChem pillows



3.10 Sample cells

A set of sample cells are shipped with each photometric instrument. The same solution in both cells gives the same absorbance (within ±0.002 Abs for properly matched cells). For more information, refer to Match the sample cells on page 21.

For accurate results, use only the sample cells specified in each procedure. Due to differences in cell path lengths, sample cell substitution introduces bias in test results. For example, 25.4-mm (1-inch) square cells have a path length approximately 8% longer than 25.4-mm (1-inch) round cells. Substitution of round cells for square cells introduces a bias in the reading.

3.10.1 Orientation of the sample cells

To minimize measurement variability when a particular cell is used, always orient the cell in the same manner before it is put into the cell holder. The fill marks on the cells can be used as orientation guides to position the cells.

3.10.2 Maintain the sample cells

Keep the sample cells in the supplied boxes to protect them from scratches and breakage. After use, empty and clean the sample cells. Do not leave color solutions in the sample cells for extended periods of time.

3.10.3 Clean the sample cells

⚠ CAUTION

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

⚠ CAUTION

Chemical exposure hazard. Dispose of chemicals and wastes in accordance with local, regional and national regulations.

Most laboratory detergents are used at recommended concentrations. Neutral detergents, such as Liquinox, are safer to use when regular cleaning is necessary. To decrease the cleaning times, increase the temperature or use an ultrasonic bath. To complete the cleaning, rinse a few times with deionized water and then let the sample cell air dry. Sample cells may also be cleaned with acid, followed by a thorough rinse with deionized water.

Note: *Always use acid to clean sample cells that were used for low-level metal tests.*

Special cleaning methods are necessary for individual procedures. When a brush is used to clean sample cells, take extra care to avoid scratches on the interior surfaces of the sample cells.

3.10.4 Match the sample cells

The sample cells supplied with the spectrophotometer instrument are distortion-free. Nicks and scratches from movement may cause an optical mismatch between two sample cells and introduce error into the test results. To prevent this type of error, optically match the sample cells.

Note: *Refer to the spectrophotometer user manual for the specific steps necessary to select wavelengths and set the instrument to zero.*

1. Set the instrument power switch to on. Make sure that the Display Lock is off or the Reading mode is set to Continuous.
2. Select a wavelength of 510 nm or the wavelength to be used for the test.
3. Pour at least 10 mL (25 mL for 25-mL cells) of deionized water into each of the two sample cells.
4. Put one sample cell into the cell holder with the fill mark toward the user.
5. Set the instrument to zero.
6. Put the other sample cell into the cell holder with the fill line toward the user.
7. Let the value stabilize and then read the absorbance. Record the resulting absorbance.
8. Turn the cell 180° and do step 6 again. Try to get an absorbance value within ±0.002 Abs of the first cell. Record the orientation of the cell.
   If the sample cells cannot be matched to within ±0.002 Abs, they can still be used if an adjustment is made for the difference. For example, if the second cell reads 0.003 absorbance units higher than the first cell, adjust future readings (when these two cells are used). Subtract 0.003 absorbance units (or the equivalent concentration) from the reading. Likewise, if the second cell reads –0.003 absorbance units, add 0.003 absorbance units to the reading.

3.11 Other apparatus

3.11.1 Boiling aids

Boiling is necessary for some procedures. Under some conditions, bumping may occur and cause sample loss or injury. Bumping is caused by the sudden, almost explosive, conversion of water to steam as it is heated. Use of a boiling aid, such as boiling chips (1483531), decreases bumping.
Make sure that the boiling aids do not contaminate the sample. Do not use boiling aids (except glass beads, 259600) more than once. Use a large sufficient flask or beaker to give significant head space above the solution. Loosely cover the sample during boiling to prevent splash, reduce the chance of contamination and minimize sample loss.

Individual procedures recommend the specific boiling aid to use.

3.12 Achieve accuracy in measurement

3.12.1 Pipets and graduated cylinders

⚠CAUTION

Chemical exposure hazard. The top of the pipet is open. Always use a pipet filler bulb to pull the liquid into the pipet.

When smaller sample quantities are used, the accuracy of measurements becomes increasingly important. Figure 9 shows the correct way to read the sample level with the meniscus formed when the liquid wets the graduated cylinder or pipet walls.

Before use, rinse the pipet or cylinder two or three times with the sample to be tested. Use a pipet filler or pipet bulb to pull the sample into the pipet. When a pipet is filled, keep the tip of the pipet below the surface of the sample as the sample is pulled into the pipet. Serological pipets have marks that show the volume of liquid delivered by the pipet. The marks may extend to the tip of the pipet or may be only on the straight portion of the tube.

If the marks are only on the straight part of the tube:

1. Fill the serological pipets to the zero mark.
2. To discharge the sample, drain the sample until the meniscus is level with the necessary mark.

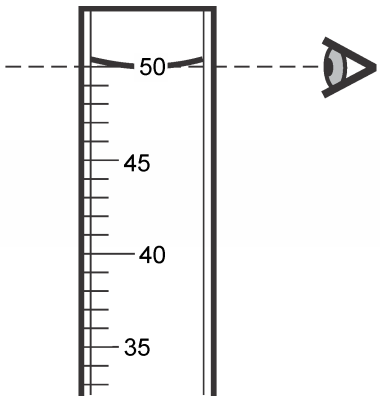
If the serological pipet has marks that extend to the tip of the pipet:

1. Fill the pipet to the applicable volume.
2. Drain all of the sample from the pipet.
3. For accurate measurements, use a pipet filler to blow the sample out of the pipet tip.

Volumetric (transfer) pipets have a bulb in the middle and a single ring above the bulb to show the volume of liquid when it is filled to the mark. To discharge a volumetric pipet, hold the tip of the pipet at a slight angle against the container wall and drain. Do not discharge the solution still in the tip of the pipet after it is drained. Volumetric pipets are made to keep a small amount of sample in the pipet tip.

If droplets of the sample hold to the walls of the pipet, the pipet is dirty and will not supply the correct amount of sample. Fully clean the pipet with a laboratory detergent or cleaning solution and then rinse several times with deionized water.

Figure 9 Read the meniscus



3.12.2 Pour-Thru™ Cell

The Pour-Thru Cell is an optional accessory that increases accuracy and makes measurements more convenient for the rapid liquid methods. Methods that use 25-mL samples and sample cells can use the Pour-Thru Cell if specified in the procedure. The Pour-Thru Cell cannot be used with 10-mL sample sizes and reagents. The Pour-Thru Cell cannot be used directly with a method unless it is specified in the procedure. For more information, refer to the photometer user manual.
Refer to the photometer user manual for installation and operation instructions.

- Pour the solution into the funnel of the installed Pour-Thru Cell Module. Do not spill solution on the instrument.
- The funnel height and orientation may be adjusted. The funnel height increases the speed of the sample flow through the cell. The higher the funnel, the faster the flow.
- To minimize air bubbles, adjust the funnel so that it drains fully with the final level of liquid in the tube about 5 cm (2 inches) below the tip of the funnel.
- Take instrument readings after the solution has stopped flowing through the cell.
- Always rinse the cell thoroughly with deionized water after each series of tests or as often as specified in the procedure.

Occasionally, remove the Pour-Thru Cell to look for any accumulation of film on the windows. If the windows are not clear (have a film), soak the cell in a detergent bath and rinse thoroughly with deionized water.

Section 4 Chemical analysis

4.1 Sample collection preservation and storage

Correct sampling and storage are critical for accurate testing. Sampling devices and containers must be thoroughly cleaned to prevent carryover from previous samples. Preserve the sample with the test-specific information about sample preservation.

4.1.1 Collect water samples

Use a clean container. Rinse the container several times with the water to be sampled, and then take the sample. Document the location and procedure used for each sample taken. For example:

From a tap—Take samples as close as possible to the source of the supply. This decreases the influence of the distribution system on the sample. Make sure that there is sufficient water to flush the system. Fill sample containers slowly with a gentle stream to avoid turbulence and air bubbles.

From a well—Let the pump run long enough to pull fresh groundwater into the system. Collect a sample from a tap near the well.

From open waters—Take the sample as near the middle of the body of water as is practical, at least several feet from the shore or edge of the tank. Take the sample under the surface of the water. When a capped container is used, submerge it before the cap is removed.

4.1.1.1 Types of containers

Different containers are recommended for specific parameters.

- Polypropylene and Polyethylene
- Quartz or TFE (tetrafluoroethylene, Teflon®)—higher quality and price
- Glass—Glass supplies a good general-purpose container. Do not use soft-glass containers to collect samples to be tested for metals in the µg/L range.

To find silver, put samples in dark containers such as amber or brown glass.

Acid wash the sample containers to fully clean them before use.

4.1.1.2 Acid washing

If a procedure suggests acid washing, do the steps that follow:

1. Clean the glassware or plasticware with laboratory detergent. Phosphate-free detergent is best. To find phosphates, always use phosphate-free detergent.
2. Rinse well with tap water.
3. Rinse with a 1:1 hydrochloric acid solution or a 1:1 nitric acid solution. To test for lead or other metals, nitric acid is best.
4. Rinse well with deionized water. For chromium, 12–15 rinses may be necessary. To test for ammonia and Kjeldahl nitrogen, make sure that the rinse water is ammonia-free.
5. Air dry the container. Protect the glassware from fumes and other sources of contamination during storage.

Use chromic acid or chromium-free substitutes to remove organic deposits from glass containers. Afterward, rinse thoroughly with water to remove all traces of chromium.

Do not use metal contaminants from containers, distilled water or membrane filters.

4.1.1.3 Sample splits

Samples must often divided into separate containers for intra- or inter-laboratory use in studies, confirmation, alternative techniques or to keep additional sample for reference and stability studies.

It is very important that sample be divided done correctly:

- Collect a large volume of sample in a single container and transfer to smaller containers. Do not fill the smaller containers individually from the water source.
- Fully mix samples that contain particulates or solids before they are divided so that all the samples are homogeneous.
- If it is necessary to filter the sample before analysis or storage, filter all the sample before it is divided.
- Use the same kind of container for all the samples.
- Analyze biologically active splits on the same day or as close to the same day as is possible.
- Preserve all splits in the same way. If this is not done, fully record the differing methods.
- When the sample is to be tested for volatile contaminants, fill containers so that they overflow and then put on a cap carefully. Do not leave any head space or air in the container.

4.1.2 Storage and preservation

Because chemical and biological processes continue after collection, analyze the sample as soon as possible. This also reduces the chance for error and minimizes labor. When an immediate analysis is not possible, preserve the sample. Preservation methods include pH control, chemical addition, refrigeration and freezing.

Comparison of international drinking water and FDA bottled water guidelines gives an overview of preservation methods and holding times for specific procedures.

Preserve aluminum, cadmium, chromium, cobalt, copper, iron, lead, nickel, potassium, silver and zinc samples for at least 24 hours with the steps that follow.

1. Add approximately 2.5 mL Nitric Acid 1:1 solution (254049) per 1 L of sample until a pH less than 2 is reached.
2. Use pH indicator paper or a pH meter to make sure that the pH is 2 or less. Add additional pillows if necessary.
3. Adjust the sample pH before analysis. Increase the pH to 4.5 with Sodium Hydroxide Standard Solution, 1 N or 5 N.

4.1.2.1 Sample preservation

Comparison of international drinking water and FDA bottled water guidelines gives an overview of preservation methods and holding times* for specific procedures. Refer to Table 7.

Table 7 Necessary containers, preservation techniques and holding times

Parameter name	Container[1]	Preservation[2,3]	Maximum holding time[4]
Bacterial tests			
Coliform, fecal and total	P, G	Cool, 4 °C to less than 10 °C, 0.0008% $Na_2S_2O_3$	8 hours
Fecal streptococci	P, G	Cool, 4 °C to less than 10 °C, 0.0008% $Na_2S_2O_3$	8 hours
Aquatic toxicity tests			
Toxicity, acute and chronic	P, G	Cool, 4 ≤ 6 °C	36 hours

* This table was adapted from Table II in the Code of Federal Regulations, Vol 77, No. 97/Friday, May 18, 2012/Rules and Regulations, pages 29806–29809. Most organic tests are not included.

Table 7 Necessary containers, preservation techniques and holding times (continued)

Parameter name	Container[1]	Preservation[2,3]	Maximum holding time[4]
Chemical tests			
Acidity	P, G	Cool, 4 ≤ 6 °C	14 days
Alkalinity	P, G	Cool, 4 ≤ 6 °C	14 days
Ammonia	P, G	Cool, 4 ≤ 6 °C H_2SO_4 to pH less than 2	28 days
Biochemical oxygen demand (BOD)	P, G	Cool, 4 ≤ 6 °C	48 hours
Biochemical oxygen demand, carbonaceous (CBOD)	P, G	Cool, 4 ≤ 6 °C	48 hours
Boron	P, PFTE or quartz	HNO_3 to pH less than 2	6 months
Bromide	P, G	Not necessary	28 days
Chemical oxygen demand (COD)	P, G	Cool, 4 ≤ 6 °C, H_2SO_4 to pH less than 2	28 days
Chloride	P, G	Not necessary	28 days
Chlorine, total residual	P, G	Not necessary	Analyze immediately
Color	P, G	Cool, 4 ≤ 6 °C	48 hours
Cyanide, total and amenable to chlorination	P, G	Cool, 4 ≤ 6 °C, NaOH to pH higher than 12, 0.6 g ascorbic acid[5]	14 days[6]
Fluoride	P	Not necessary	28 days
Hardness	P, G	HNO_3 to pH less than 2, H_2SO_4 to pH less than 2	6 months
Hydrogen ion (pH)	P, G	Not necessary	Analyze immediately
Kjeldahl and organic nitrogen	P, G	Cool, 4 ≤ 6 °C, H_2SO_4 to pH less than 2	28 days
Metals[7]			
Chromium VI	P, G	Cool, 4 ≤ 6 °C, $(NH_4)_2SO_4$ buffer to pH 9.3 – 9.7	28 days[8]
Mercury	P, G	HNO_3 to pH less than 2	28 days
Metals, except boron, chromium VI and mercury	P, G	HNO_3 to pH less than 2	6 months
Nitrate	P, G	Cool, 4 ≤ 6 °C	48 hours
Nitrate-nitrite	P, G	Cool, 4 ≤ 6 °C, H_2SO_4 to pH less than 2	28 days
Nitrite	P, G	Cool, 4 ≤ 6 °C	48 hours
Oil and grease	G	Cool, 4 ≤ 6 °C, HCl or H_2SO_4 to pH less than 2	28 days
Organic Carbon	P, G	Cool, 4 ≤ 6 °C, HCl or H_2SO_4 or H_3PO_4 to pH less than 2	28 days
Orthophosphate	P, G	Filter immediately; Cool, 4 ≤ 6 °C	48 hours
Oxygen, dissolved probe	G Bottle and top	Not necessary	Analyze immediately
Winkler	G Bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G only	Cool, 4 ≤ 6 °C, H_2SO_4 to pH less than 2	28 days

Table 7 Necessary containers, preservation techniques and holding times (continued)

Parameter name	Container[1]	Preservation[2,3]	Maximum holding time[4]
Phosphorus, elemental	G	Cool, 4 ≤ 6 °C	48 hours
Phosphorus, total	P, G	Cool, 4 ≤ 6 °C, H_2SO_4 to pH less than 2	28 days
Residue, Total	P, G	Cool, 4 ≤ 6 °C	7 days
Residue, Filterable	P, G	Cool, 4 ≤ 6 °C	7 days
Residue, Nonfilterable (TSS)	P, G	Cool, 4 ≤ 6 °C	7 days
Residue, Settleable	P, G	Cool, 4 ≤ 6 °C	48 hours
Residue, Volatile	P, G	Cool, 4 ≤ 6 °C	7 days
Silica	P, PFTE or quartz	Cool, 4 ≤ 6 °C	28 days
Specific Conductance	P, G	Cool, 4 ≤ 6 °C	28 days
Sulfate	P, G	Cool, 4 ≤ 6 °C	28 days
Sulfide	P, G	Cool, 4 ≤ 6 °C, add zinc acetate plus sodium hydroxide to pH higher than 9	7 days
Sulfite	P, G	Not necessary	Analyze immediately
Surfactants	P, G	Cool, 4 ≤ 6 °C	48 hours
Temperature	P, G	Not necessary	Analyze immediately
Turbidity	P, G	Cool, 4 ≤ 6 °C	48 hours

1 Polyethylene (P), glass (G) or PTFE Teflon

2 Sample preservation should be completed immediately upon sample collection. For composite chemical samples, each portion should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each portion, chemical samples may be preserved at 4 ≤ 6 °C until compositing and sample splitting is completed.

3 When any sample is to be shipped by common carrier or sent through United States mail, it must comply with the Department of Transportation Hazardous Material Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for making sure of such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation have found that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or higher); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or higher); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or higher); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

4 Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that the specific types of samples under study are stable for the longer time and the permitee has received a variance from the Regional Administrator under §136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to keep sample stability. Refer to §136.3(e) for details. The term “analyze immediately” usually means within 15 minutes or less after sample collection.

5 Should only be used in the presence of residual chlorine.

6 Maximum holding time is 24 hours when sulfide is present. Optionally, all samples may be analyzed with lead acetate paper before pH adjustments to find sulfide. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to pH 12.

7 Samples should be filtered immediately on-site before preservative is added for dissolved metals.

8 From 40 CFR 136.3 - To get the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. Dissolve 38 g ammonium sulfate in 75 mL ASTM type I water and add 6.5 mL of ammonium hydroxide. Dilute to 100 mL with ASTM type I water. Adjust to pH 9 – 9.5 with the buffer and correct for volume additions.

4.1.2.2 Correct for volume additions

When a large volume of preservative or neutralizer is used, account for dilution by the acid that was added to preserve the sample and/or the base used to adjust the pH to the range of the procedure. Do the steps that follow to make this correction:

1. Find the volume of the initial sample, the volume of acid and base added and the total final volume of the sample.
2. Divide the total volume by the initial volume.
3. Multiply the test result by the result of step 2.

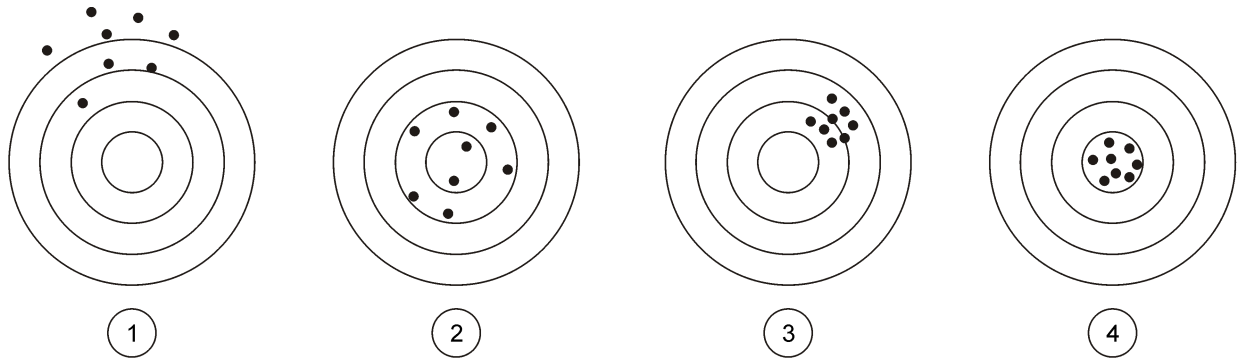
   Example:
   A 1 L sample was preserved with 2 mL of nitric acid. It was neutralized with 5 mL of 5 N sodium hydroxide. The result of the analysis procedure was 10.00 mg/L. What is the volume correction factor and correct result?

   1. Total volume = 1000 mL + 2 mL + 5 mL = 1007 mL
   2. 1007 ÷ 1000 = 1.007 = volume correction factor
   3. 10 mg/L × 1.007 = 10.07 mg/L = correct result

4.1.3 About accuracy and precision

Accuracy defines how near a test result is to the true value. Precision defines how near repeated measurements are to each other. Although precise results suggest accuracy, they can be inaccurate. Both the accuracy and the precision of test results can be evaluated with standard additions or standard solutions. Refer to Figure 10.

Figure 10 Precision vs accuracy



1 Not accurate, not precise	**3** Precise, not accurate
2 Accurate, not precise	**4** Accurate and precise

4.1.4 Standard solutions

A standard solution may be ordered as a prepared reagent or it may be made in the laboratory. It is a solution of a known composition and concentration. The accuracy of the analysis system may be identified with a standard solution in place of the sample water in a procedure.

4.1.5 Standard additions

Standard additions is a common technique to identify the accuracy of the test results. Other names are “spiking” and “known additions.” The technique can identify interferences, bad reagents, faulty instruments and incorrect procedures.

To complete the standard additions technique, add a measured small amount of a standard solution to the sample and do the test again. Use the same reagents, equipment and technique. The result should be about 100% recovery. If not, there is an identifiable problem.

If the standard additions technique is satisfactory for the test, a standard additions method section will be in the procedure under Accuracy Check. Complete the detailed instructions given.

If the result is approximately 100% recovery for each addition, everything is satisfactory and the results are correct.

If the result is not approximately 100% recovery for each addition, a problem is present. To identify if the cause is an interference, do the standard additions technique again with deionized water as the sample. If the result is approximately 100% recovery for each addition, an interference exists.

If the results show good recoveries with the deionized water, use this checklist to find the problem:

1. Make sure that the steps in the procedure are done correctly:
   a. Are the correct reagents used in the correct order?
   b. Is the correct time used to let the color develop?
   c. Is the correct glassware used?
   d. Is the glassware clean?
   e. Does the test need a specific sample temperature?
   f. Is the sample pH in the correct range?

   Refer to the written procedure to answer these questions.
2. Examine the performance of the instrument with the instructions in the user manual.
3. Examine the reagents. Repeat the standard additions technique with new, fresh reagents. If the results are good, the original reagents were faulty.
4. If nothing else is wrong, the standard is almost certainly defective. Do the standard additions technique again with a new standard.

If the problem is still not known, contact technical support. Contact information is provided on the website for all countries.

4.1.6 Troubleshoot a test when the results are in doubt

If the results from any chemistry are in doubt, do the steps that follow to troubleshoot.

1. Do a proof-of-accuracy check. Take a standard solution, which has a known concentration, through the same steps as the original sample. Include sampling and storage, digestion and colorimetric determination, if applicable. If the results of the standard solution check are correct, go to step 4. If there is a variation in the expected results, go to step 2.
2. If the standard solutions check is not the same as the expected results, examine the instrument set-up and method procedure with the steps that follow:
   a. Make sure that the correct program number for the test is selected.
   b. Make sure that the units of concentration of the standard are the same as the units shown. (One of the alternative forms of the analyte may be in the display.) For example: Molybdenum may be shown as Mo instead of MoO_4.
   c. Make sure that the sample cells specified in the procedures are used.
   d. Make sure that the reagents are correct for the sample size being analyzed.
   e. Make sure that the reagent blank value saved is for the current procedure. It may be from a previous lot of reagents and therefore not representative of the current reagent lot.
   f. Make sure that the calibration curve adjustment (Standard Adjust) is currently in use. The factory-stored default calibration should be used initially to examine the standard.
   g. Make sure that the dilution factor option is correct.

If the instrument setup is correct and the method procedure specifics are completed correctly, go to step 3.

3. If the standard solution check does not match the expected results, examine the reagents used in the test and the analytical technique with the steps that follow:

   a. Find the age of the reagents used in the test. Many factors affect reagent shelf life (i.e., storage temperature, storage conditions, microbial contamination). Replace suspect reagents and do the standards check again.
   b. Do a deionized or distilled water blank through the full process (include sampling and storage, digestion and colorimetric determination). Some chemicals will add a small amount of color to a test. This is typical. However, color development higher than 10% of the range of the test may show a problem with one of the reagents or the dilution water.
   c. To troubleshoot the procedure, delete the parts one by one. First, do the standard solution, leave out preservation and storage, and do only digestion and colorimetry. If this analysis is correct, examine the procedure used to keep the sample. Make sure that it is the procedure prescribed for the selected parameter. If the sample is acidified for storage, make sure that the correct acid is used and the sample is adjusted to the proper pH level before the sample is examined.

   If the standards check is still incorrect, do the standard on just the colorimetry. If the results are correct, examine the digestion procedure. Make sure that the amount of reagents used and the pH after the digestion are correct for the procedure. Refer to the procedure for the parameter in question.

4. If the standard solution gives a correct value, but the results of the sample measurement are questionable, there may be an interference in the sample. To look for an interference:

   a. Spike the sample. Use a standard addition test instead of a standard solution test to include any possible interferences.
   b. To test cells that contain fresh sample water, add an amount of standard equal to two times the concentration of the sample.
   c. Do both samples with the same reagents, instruments and technique. The spiked sample should show an increase equal to the amount of standard added.
   d. Calculate percent recovery as shown below. Ideally, the results should be 100%. The results from 90 to 110% are acceptable. Refer to the procedure notes for possible interferences and ways to prevent them.
   e. Do a series of dilutions on the sample. Make sure that the sample is within the range of the test. An out-of-range sample for the method may give erroneous results because of under- or over-development of the color, too much turbidity or even sample bleaching. Do a series of dilutions to look for this possibility.
   f. If the cause of the interference cannot be found, dilute the sample past the point of interference. This is often the most economical and efficient way to get the correct result. If it is not possible to dilute out an interference without diluting out the parameter to be measured, use a different method, such as a different chemistry or an ion-selective electrode to measure the parameter.

4.1.6.1 Calculate the percent recovery

To calculate the percent recovery:

1. Measure the unknown sample concentration.
2. Calculate the theoretical concentration of the spiked sample:
   Theoretical concentration = $(C_u \times V_u) + (C_s \times V_s) \div V_u + V_s$

   Where:

   C_u = measured concentration of the unknown sample

   V_u = volume of the unknown sample

C_s = concentration of the standard

V_s = volume of the standard

3. Measure the spiked sample concentration.
4. Divide the spiked sample concentration by the theoretical concentration and multiply by 100.
   For example:
   A sample was tested for manganese and the result was 4.5 mg/L. A separate 97-mL portion of the same sample was spiked with 3 mL of a 100 mg/L standard solution of manganese. This spiked solution was examined again for manganese with the same method. The result was 7.1 mg/L.

   The theoretical concentration of the spiked sample is:

   (4.5 mg/L × 97 mL) + (100 mg/L × 3 mL) ÷ 97 mL + 3 mL = 7.4 mg/L

   The percent spike recovery is:

   (7.1 mg/L ÷ 7.4 mg/L) x 100 = 96%

4.1.6.2 USEPA calculation

The USEPA uses a more stringent calculation requirement for percent recovery. This formula calculates the percent recovery only for the standard added to the spiked sample and gives a lower value than the above calculation. A complete explanation for the USEPA formula is shown in USEPA Publication SW-846. The USEPA percent recovery formula is:

$\%R = 100 (X_s – X_u) \div K$

Where:

X_s = measured value of the spiked sample

X_u = measured value for the unspiked sample, adjusted for the dilution of the spike volume

K = known value of the spike in the sample

For example:

A sample measures 10 mg/L. A separate 100-mL portion of the sample was spiked with 5 mL of a 100-mg/L standard solution. The spiked solution was measured by the same method as the original sample. The result was 13.7 mg/L.

X_s = 13.7 mg/L

X_u = (10 mg/L × 100 mL) ÷ 105 mL = 9.5 mg/L

K = (5 mL × 100 mg/L) ÷ 105 mL = 4.8 mg/L

%R = (100 × (13.7 mg/L – 9.5 mg/L)) ÷ 4.8 mg/L = 88%

Acceptable percent recovery values are 80–120%.

4.1.7 Adjust the standard curve

Spectrophotometers typically have many programs permanently installed in memory. Many programs include a pre-programmed calibration curve. Each curve is the result of an extensive calibration completed under ideal conditions and is normally sufficient for most testing. Deviations from the curve can occur from compromised testing reagents, defective sample cells, incorrect test procedure, incorrect technique or other correctable causes. Interfering substances or other causes may be beyond the control of the analyst.

The pre-programmed curve may not be convenient in the situations that follow:

- Tests are done where the reagents are highly variable from lot to lot.
- Tests are done where frequent calibration curve checks are necessary.
- Tests are done where samples give a consistent test interference.
- Think about the questions that follow before the calibration curve is adjusted:
  - Will future test results be better when the curve is adjusted?
  - Are interfering substances consistent in all the samples tested?

- Estimated detection limit, sensitivity, precision and test range information provided with the procedure may not apply to an adjusted curve calibration.

The calibration curves can be adjusted with the steps found in the test procedure. Generally, add test reagents to a blank and standard solution. It is important to do the procedure carefully. After the adjustment, it is wise to do standard solutions of several concentrations to make sure that the adjusted curve is satisfactory. Do standard additions on typical samples to find out if the adjusted curve is acceptable.

To adjust a measurement is a two-step process. First, the instrument measures the sample with the pre-programmed calibration. Second, the instrument multiplies this measurement by an adjustment factor. The factor is the same for all concentrations. The instrument remembers the factor until the program is exited and shows the standard adjustment icon when it is used. To return to the pre-programmed curve at any time, select the original stored program from the main menu.

4.2 Interferences

Interferences are contaminants in a sample that can cause changes in color development, turbidity or unusual colors and odors, and thereby make errors in the results. A list of common interferences is included in each procedure. Reagents are formulated to remove many interferences. To remove other interferences, pretreat the sample as instructed in the procedure.

Test strips are available for many of the common interferences. These test strips can be used to screen samples for the presence of interferences.

1. Repeat the test on a sample diluted with deionized water. Refer to Sample dilution on page 18.
2. Correct the results for the dilution and compare them with those from the original test.
3. If they are significantly different, make a second dilution and compare it against the first.
4. Repeat the dilutions until the same result (after volume corrections) is achieved twice in succession.

For more information on interferences, refer to Standard additions on page 29. The APHA Standard Methods book (an excellent reference for water analysis) also shows interferences in the general introduction section.

4.2.1 pH interference

Chemical reactions are often pH dependent. Reagents contain buffers to adjust the pH of the sample to the correct range. However, the reagent buffer may not be strong enough for samples that are highly buffered or have an extreme pH. The sampling and storage section of each procedure gives the pH range for that test.
Before the test, adjust the sample to the proper pH as instructed in the procedure or do the steps that follow:

1. Measure the pH of the analyzed sample with a pH meter.
   Note: *Use pH paper to test for chloride, potassium or silver to avoid contamination.*
2. Prepare a reagent blank with deionized water as the sample. Add all reagents specified in the procedure. Timer sequences, etc., may be ignored.
3. Mix well.
4. Measure the pH of the reagent blank with a pH meter.
5. Compare the pH values of the analyzed sample with the reagent blank.
6. If there is little difference in the values of the analyzed sample and the reagent blank, the pH interference is not the problem. Do the accuracy check for the specific procedure to more clearly identify the problem.

7. If there is a large difference between the value of the analyzed sample and the reagent blank, adjust the sample pH to the value of the reagent blank. Adjust the sample pH to this same pH for all future samples before analysis.

   - Use the applicable acid, usually nitric acid, to lower the pH.
   - Use the applicable base, usually sodium hydroxide, to increase the pH.

   If acid or base was added, adjust the final result for any dilution that was caused. Refer to Correct for volume additions on page 29.
8. Analyze the sample as done previously.
9. Some purchased standards may be very acidic and do not work directly with test procedures. Adjust the pH of these standards as described previously. Adjust the final concentration of the standard for the dilution. The standard solutions suggested in the procedures are formulated so that no pH adjustment is necessary.

4.3 Method performance

4.3.1 Estimated detection limit (EDL)

Ranges for chemical measurements have limits. The lower limit is important because it identifies whether a measurement is different from zero. Many experts disagree about the definition of this detection limit and find that it can be difficult. The Code of Federal Regulations (40 CFR, Part 136, Appendix B) provides a procedure to find the Method Detection Limit (MDL). The MDL is the lowest concentration that is different from zero with a 99% level of confidence. A measurement below this MDL is highly suspect.

The MDL is not fixed. It is different for each reagent lot, instrument, analyst, sample type, etc. Therefore, a published MDL may be a useful guide, but is only accurate for a specific set of circumstances. Each analyst should find a more accurate MDL for each specific sample matrix with the same equipment, reagents and standards that will routinely be used for measurements.

A sensitivity value (concentration change equivalent to an absorbance change of 0.010 abs) is provided as an estimate of the lower detection limit of each test. The sensitivity value may be used as an EDL for the purposes of MDL determination. It is a good starting concentration when a MDL is to be found.
Note: *Do not use the EDL as the MDL.*

The conditions for MDL determination must be exactly the same as the conditions used for analysis. The EDL may be useful to the analyst as a starting point when a MDL is to be found or as a way to compare methods. Measurements below the EDL may also be valuable because they can show a trend, show the presence of analyte and/or provide statistical data. However, these values have a large uncertainty.

4.3.2 Method detection limit (MDL)

This method is in accordance with the USEPA definition in 40 CFR, Part 136, Appendix B in the 7-1-94 edition. The USEPA defines the method detection limit (MDL) as the minimum concentration that can be found with a 99% level of confidence that the true concentration is higher than zero. Since the MDL is different from analyst to analyst, it is important that the MDL be found under actual operating conditions.
The procedure to find the MDL is based on replicate analyses at a concentration 1 to 5 times the estimated detection limit. The MDL value is calculated from the standard deviation of the replicate study results multiplied by the appropriate **t** value for a 99% confidence interval. For this definition, the MDL does not account for variation in sample composition and can only be achieved under ideal conditions.

1. Make an estimate of the detection limit. Use the sensitivity value stated in the Method performance section of the analysis procedure.
2. Prepare a laboratory standard of the analyte, 1 to 5 times the estimated detection limit, in deionized water that is free of the analyte.

3. Make an analysis of at least seven portions of the laboratory standard and record each result.
4. Calculate the average and the standard deviation(s) of the results.
5. Calculate the MDL with the appropriate **t** value (Table 8) and the standard deviation value:

   MDL = **t** × s

Table 8 Test portions and t values

Number of test portions	t value
7	3.143
8	2.998
9	2.896
10	2.821

For example:
The EDL to measure iron with an iron test is 0.003 mg/L. An analyst accurately prepared 1 L of a 0.010 mg/L (about 3x the EDL) laboratory standard with a mixture of a 10-mg/L iron standard in iron-free deionized water.

Eight portions of the standard were examined with the FerroZine method. The results are shown in Table 9.

Table 9 Samples and results

Sample #	Result (mg/L)
1	0.009
2	0.010
3	0.009
4	0.010
5	0.008
6	0.011
7	0.010
8	0.009

Use a calculator program. The average concentration = 0.010 mg/L and the standard deviation (s) = 0.0009 mg/L.

Based on the USEPA definition, calculate the MDL:

MDL for iron test = 2.998 (**t**) x 0.0009 (s)

MDL = 0.003 mg/L (the same as the initial estimate)

Note: *Occasionally, the calculated MDL may be very different from the estimate of the detection limit. To test how reasonable the calculated MDL is, repeat the procedure with a standard near the calculated MDL. The average result calculated for the second MDL derivation should agree with the initial calculated MDL. Refer to 40 CFR, Part 136, Appendix B (7-1-94), pages 635–637 for detailed procedures to make sure that the MDL determination is correct.*

1. Put a laboratory blank (that contains deionized water without analyte) through the test procedure to to make sure that the blank measurement is less than the calculated MDL.
2. If the blank measurement is near the calculated MDL, repeat the MDL procedure with a separate blank for analysis for each portion of standard solution analyzed.
3. Subtract the average blank measurement from each standard and use the corrected standard values to calculate the average and standard deviation used in the MDL.

4.3.3 Precision

Every chemical measurement has some degree of uncertainty. The quality of the entire calibration curve determines the precision.

Uncertainty in chemical measurements may be due to systematic errors and/or random errors. A systematic error is a mistake that is always the same for every measurement made. For example, a blank can add to each measurement for a specific compound, and gives consistently high results (a positive bias). Random errors are different for every test and can add a positive or a negative variation in response. Random errors are most often caused by variation in analytical technique. Even with reliable reagents developed to prevent systematic errors, response variation occurs in all chemical measurements.

4.3.4 Estimate the precision

The method performance section in each procedure gives an estimate of test precision. Most procedures use a replicate analysis estimate, based on real data. For precision found in this manner, the 95% confidence interval of the distribution is reported.

In replicate analysis, the chemist prepares a specific concentration of the analyte in a deionized water matrix. The standard is analyzed seven individual times on a single instrument. The standard deviation is calculated and the 95% confidence interval of the distribution is reported in the method. The reported value gives an estimate of the “scatter” of results at a particular point in the calibration curve.

Precision estimates are based on a deionized water matrix. Precision on real samples with varying matrices can be quite different from these estimates.

If the concentration achieved from the use of a standard solution is not as expected or when the results are in doubt, refer to Troubleshoot a test.

4.3.5 Sensitivity

The definition of the sensitivity of a method is a change in concentration (ΔConcentration) for a 0.010 change in absorbance (ΔAbs).

Use sensitivity when different methods are compared. For example, when three methods are used to find iron (Table 10).

Table 10 Concentration and absorbance

Iron analysis method	Portion of curve	ΔAbs	ΔConcentration
FerroVer	Entire range	0.010	0.022 mg/L
FerroZine	Entire range	0.010	0.009 mg/L
TPTZ	Entire range	0.010	0.012 mg/L

Notice that the FerroZine method has the larger sensitivity of the three methods because it measures the smallest change in concentration. The technical definition of sensitivity comes from a calibration curve with Abs on the x-axis and concentration on the y-axis.

- If the calibration is a line, the sensitivity is the slope of the line multiplied by 0.010.
- If the calibration is a curve, the sensitivity is the slope of the tangent line to the curve at the concentration of interest multiplied by 0.010.

The sensitivity value is also used as an estimate of the lower limit of the test. The value may be used as a starting point to find the MDL.

4.4 Prepare a calibration curve

Note: *Calibration curves are recommended when tests are done on different instruments or where necessary by a regulator.*

1. Prepare five or more standards of known concentration that cover the expected range of the test.
2. Do tests as described in the procedure on each prepared standard.
3. Pour the customary volume of each known solution into a separate clean sample cell of the type specified for the instrument.
4. Select the proper wavelength. Standardize (zero) the instrument with an untreated water sample or a reagent blank as specified in the procedure instructions.
5. Measure and record the absorbance of the known solutions within the time constraints specified in the procedure. To use absorbance vs. concentration, refer to Absorbance versus concentration calibration on page 37.

4.4.1 Absorbance versus concentration calibration

1. If absorbance values are measured, plot the results on linear graph paper.
   a. Plot the absorbance value on the y-axis and the concentration on the x-axis.
   b. Plot increasing absorbance values from bottom to top.
   c. Plot increasing concentration values from left to right.
   Values of 0.000 absorbance units and 0 concentration will start at the bottom left corner of the graph. A calibration table can be extrapolated from the curve or the concentration values can be read directly from the graph. Another alternative is to find an equation for the line with the slope and y-intercept.
2. As an alternative, use the user program software in the spectrophotometer or a curve-fitting program (such as a spreadsheet software) to calculate the calibration curve.

4.5 Adapt procedures to other spectrophotometers

Test procedures may be used with more than one spectrophotometer if calibration curves are made that convert absorbance to concentration. Regardless of the spectrophotometer used, prepare the sample and calibration standards with the procedure and use the optimum wavelength used in the procedure.
To calibrate for a given analyte, a series of standards are prepared and measured to make the calibration curve. The absorbance vs. concentration is plotted as described in Absorbance versus concentration calibration on page 37. Points on the graph are connected with a smooth line (curved or straight). If necessary, use the curve to make a calibration table.

4.5.1 Select the best wavelength

When a new procedure is developed or procedures that are sensitive to wavelength are used, select the wavelength where the instrument gives the highest absorbance. Refer to Figure 11 on page 39. Because procedures are usually developed to use the best wavelength for the test, the selection of the wavelength is not necessary for most stored procedures.

4.5.1.1 Select the best wavelength on a spectrophotometer

Note: *When available, use of the Scan function is the easiest way to find the optimum wavelength.*

1. Refer to the user manual for specific instructions for wavelength adjustments.
2. Select single wavelength adjustment.
3. Enter a wavelength in the range of interest.
   Note: *Sample color provides a good indication of which wavelength region to use.*
   - *A yellow solution absorbs light in the 400–500 nm region.*
   - *A red solution absorbs light between 500–600 nm.*
   - *A blue solution absorbs light in the 600–700 nm range.*
4. Prepare the blank and sample for analysis. Fill the applicable sample cells with the blank and the reacted sample solution.
5. Put the blank in the cell holder. Set the instrument to zero.
6. Put the prepared sample into the cell holder. Read the absorbance level.
7. Increase the wavelength so that it is at least 100 nm higher than the range of interest. Set the instrument to zero as in step 5. Measure and record the absorbance of the sample.
8. Repeat, decrease the wavelength by 50 nm. Set the instrument to zero, then measure and record the absorbance at each increment. Continue this process throughout the wavelength range of interest. Record the wavelength of highest absorbance. Refer to Table 11.

Table 11 Absorbance values at 50-nm increments

Wavelength	Absorbance
550 nm	0.477
500 nm	0.762
450 nm	0.355
400 nm	0.134

9. Adjust the wavelength to 50 nm more than the highest absorbance point on the initial search (step 8). Set the instrument to zero, then measure and record the absorbance at each increment.
   Repeat, decreasing the absorbance in 5-nm steps. Set the instrument to zero, then measure and record the absorbance at each increment. Continue until the entire range of interest is measured. Refer to Table 12.

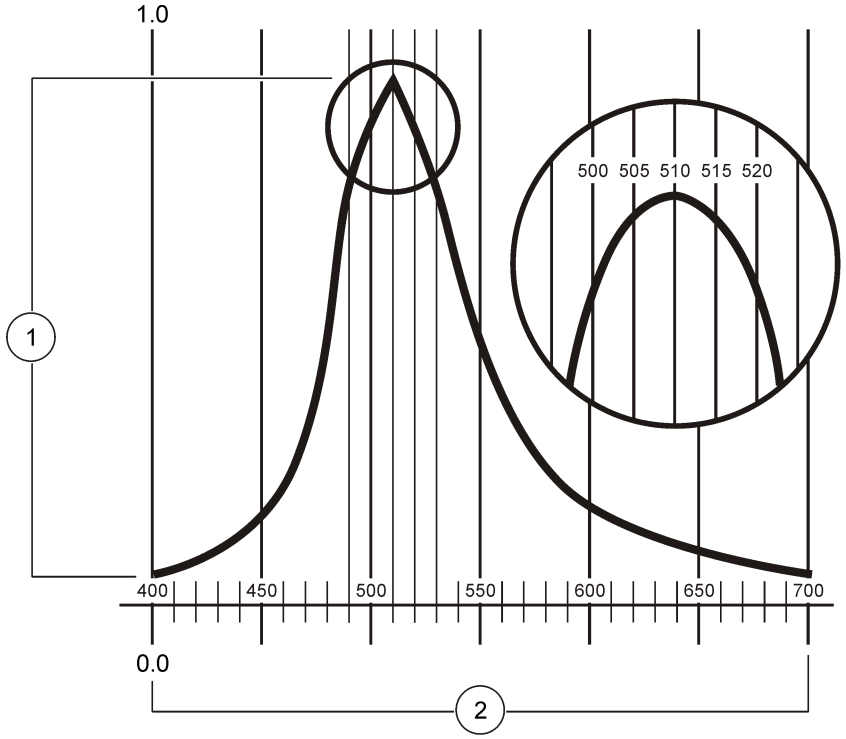
Table 12 Absorbance values at 5-nm increments

Wavelength	Absorbance
520 nm	0.748
515 nm	0.759
510 nm	0.780
505 nm	0.771
500 nm	0.771
495 nm	0.651
490 nm	0.590

Make sure that there is enough difference in absorbance between samples with low and high analyte concentrations. Measure two sample solutions that contain the expected low and high concentrations of analyte at the optimum wavelength. The change in absorbance caused by increases/decreases in concentration depends on the sensitivity of the procedure and the chemistry. Chemistries with small absorbance

changes are less sensitive, but tend to have larger ranges. Chemistries with large absorbance changes are more sensitive, but tend to have smaller ranges.

Figure 11 Select the best wavelength



1 Absorbance	**2** Wavelength (nm)

Section 5 Sample pretreatment by digestion

Several procedures use sample digestion before the total metal content is found. Digestion uses acid and heat to break organo-metallic bonds and free ions for analysis.

5.1 USEPA-approved digestions

For USEPA reporting, USEPA-approved digestions are necessary. There are two methods for metals analysis: mild and vigorous.

5.1.1 USEPA mild digestion

1. Add concentrated nitric acid to the entire sample at the time of collection. Add 5 mL of acid per liter (or quart) of sample.
2. Move 100 mL of well-mixed sample to a beaker or flask.
3. Add 5 mL of distilled 1:1 hydrochloric acid (HCl).
4. Increase the temperature of the liquid with a steam bath or hot plate until the volume has been reduced to 15–20 mL. Do not boil.
5. Use a filter to remove any insoluble material from the sample.
6. Adjust the pH of the digested sample to pH 4. Add 5.0 N Sodium Hydroxide Standard Solution a drop at a time. Mix thoroughly and examine the pH after each addition.
7. Pour the reduced sample into a 100-mL volumetric flask.
8. Use a small amount of demineralized water to rinse the beaker. Pour the rinse water into the volumetric flask.
9. Repeat the rinse process a few more times to remove all of the reduced sample from the beaker.
10. Add demineralized water to fill the volumetric flask to the 100-mL mark.
11. Use the diluted sample in the test procedure. Record the results.
12. Prepare a blank: Repeat steps 1-11 with demineralized water instead of the sample.
13. Subtract the results of the blank analysis from the results of the sample analysis.

5.1.2 USEPA vigorous digestion

For some samples mild digestion will not be sufficient. Use a vigorous digestion to make sure that all of the organo-metallic bonds are broken.

1. Use redistilled 1:1 Nitric Acid Solution to acidify the entire sample to a pH of less than pH 2. Do not filter the sample before digestion.
2. Move an appropriate sample volume into a beaker and add 3 mL of concentrated redistilled nitric acid. Refer to Table 13.
3. Put the beaker on a hot plate and evaporate to near dryness. Make sure that the sample does not boil.
4. Cool the beaker and add another 3 mL of the concentrated re-distilled nitric acid.
5. Put the cover on the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so that a gentle reflux occurs. Add additional acid, if necessary, until the digestion is complete (generally shown when the digestate is light in color or does not change color or appearance with continued refluxing).
6. Again, evaporate to near dryness (do not bake) and cool the beaker. If any residue or precipitate results from the evaporation, add redistilled 1:1 hydrochloric acid (5 mL per 100 mL of final volume). Refer to Table 13.
7. Warm the beaker. Adjust the sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution. Mix thoroughly and examine the pH after each addition.
8. Pour the reduced sample into a 100-mL volumetric flask.
9. Use a small amount of demineralized water to rinse the beaker. Pour the rinse water into the volumetric flask.

10. Repeat the rinse process a few more times to remove all of the reduced sample from the beaker.
11. Add demineralized water to fill the volumetric flask to the 100-mL mark.
12. Use the diluted sample in the test procedure. Record the results.
13. Multiply the result by the correction factor in Table 13.
14. Prepare a blank: Repeat steps 1-13 with demineralized water instead of the sample.
15. Subtract the results of the blank analysis from the results of the sample analysis.

Table 13 Vigorous digestion volumes

Expected metal concentration	Suggested sample volume for digestion	Suggested volume of 1:1 HCl	Suggested final volume after digestion	Correction factor
1 mg/L	50 mL	10 mL	200 mL	4
10 mg/L	5 mL	10 mL	200 mL	40
100 mg/L	1 mL	25 mL	500 mL	500

5.2 General Digesdahl digestion

Many samples may be digested with the Digesdahl Digestion Apparatus (2313020). It is designed to digest samples such as oils, wastewater, sludges, feeds, grains, plating baths, food and soils. In this procedure, the sample is oxidized by a mixture of sulfuric acid and hydrogen peroxide. Less than 10 minutes is necessary for the digestion of a dry sample. About 1 minute/mL is necessary for the digestion of liquid samples. The digestion is done in a special flat-bottomed, 100-mL volumetric flask. Aliquots (sample portions) are used for analysis with the colorimetric methods.

Procedures for digestion with the Digesdahl Digestion Apparatus are based on the type and form of the sample. Refer to the Digesdahl Digestion Apparatus Instruction manual supplied with the Digesdahl Digestion Apparatus.

Digesdahl digestion is a process that yields a digest that can be used to find metals, total phosphorus and total Kjeldahl nitrogen (TKN). It is faster than traditional methods, but has comparable accuracy and precision. The digest can be used with colorimetric, turbidimetric or titrimetric tests.

The procedures for the Digesdahl Digestion Apparatus vary with the sample type. Sample types include food products, feeds, grains, wastewater sludges, plating baths, plant tissues, fertilizers, beverages and oils. Most procedures use a two-phase digestion process that uses concentrated sulfuric acid and 50% hydrogen peroxide. Sulfuric acid dehydrates and chars the sample. Hydrogen peroxide is added through the capillary flow funnel to complete the decomposition. The analyst varies the volume of hydrogen peroxide used to control the digestion time (exposure to the hydrogen peroxide).

Some samples are more difficult to digest completely (e.g., resistant or refractory materials, such as nicotinic acid). Several minutes of continued peroxide digestion are necessary after clearing to get 100% nitrogen recovery. To make sure that there is complete sample digestion, think about variables such as sample size, solution temperature and sample contamination. Refer to the Digesdahl Manual (2313018) for complete information.

5.2.1 Frequently asked questions for digestion procedures

This section provides answers to common questions about digestion.

What should be done if the reading on the instrument is over-range?

The concentration range tables found in digestion procedures are only guidelines. Use a smaller analysis volume and repeat the procedure. Record the new analysis volume and use it in the calculation.

Should a reagent blank be prepared each time reagents with the same lot number are used?

To decide, first find the reading of the reagent blank. Set the instrument to zero with deionized or distilled water. If the reagent blank has an insignificant concentration reading and the reagents have the same lot number, a reagent blank does not have to be prepared every time. If the reagent blank shows a reading, analyze it daily or subtract the reading from the sample reading. If a reagent blank is not analyzed daily, set the instrument to zero with deionized water.

Does the exact sample amount and analysis volume given in each procedure need to be used?

The sample amount and the analysis volume for each procedure are only suggested guidelines. Digest any aqueous solution or suspension sample amount up to 40 mL. Less than 0.5 g of anhydrous material is necessary for solid or organic liquid samples—as a routine practice, 0.25 g of sample is used.

How can the initial amount of sample (necessary for digestion) and the analysis volume to be used be refined?

The amount of sample to be digested is a critical aspect of the digestion. The aliquot size of the digest to be used in the analysis is also very important. Tables are provided in each method to find the amount of initial sample to be digested. In order to optimize the specific test to be done, the equations that follow have been developed. Before these equations are used, refer to the manual specifications for the sample type.

To use the equations, find the approximate concentration (in ppm, mg/L or mg/kg). Next, find the range of the colorimetric test to be used (e.g., 0–50 mg/L) and the midpoint of the test range. This midpoint range is optimum but can be lowered to accommodate very low sample concentrations. To find the midpoint of the test range, subtract the lower limit of the range from the higher limit and then divide by 2.

After these determinations are finished, use the equation that follows:

$A = (B \times C \times D) \div (E \times F)$

Where:

A = approximate concentration of sample

B = midpoint of colorimetric test range

C = final volume of digest

D = final volume of analysis

E = sample amount to digest

F = analysis volume of digest

Use algebra to obtain the equations that follow:

Equation 1 is $E = (B \times C \times D) \div (A \times F)$

Equation 2 is $F = (B \times C \times D) \div (A \times E)$

Both equations contain two unknown values, E and F. Some trial and error may be necessary to get the optimum values.

Use equation 1: If the analysis is for copper, use the CuVer™ method with an initial sample that contains approximately 150 ppm Cu. The amount of sample necessary for digestion and the aliquot volume to be used can be found as follows:

Find the test range. In this example, the test range is thought to be 0–5.0 ppm and the midpoint is 2.5. When the Digesdahl system is used, the final volume of digest is 100 mL and the procedure calls for a final analysis volume of 25 mL.

Therefore:

A = 150

B = 2.5

C = 100

D = 25

E = unknown

F = unknown

Substitute values into equation (1) gives:

E = (2.5 × 100 × 25) ÷ (150 × F) or E = 41.7 ÷ F

Since CuVer Copper Reagent is pH sensitive, a small analysis volume (0.5 mL) is necessary so that pH adjustment would not be necessary.

With this in mind, a 0.5-mL analysis volume would give:

E = 41.7 ÷ 0.5 = 83.4 mL digestion sample amount

Because the maximum digestion sample amount is 40 mL for Digesdahl digestions, a 0.5-mL analysis volume is not acceptable for the range. This is where trial and error is necessary. Next, try a 5.0-mL analysis volume and the equation gives:

E = 41.7 ÷ 5.0 = 8.0 mL digestion sample amount

(Round to the nearest whole number for ease of measure.)

From the calculation, an 8.0 mL sample is digested and a 5.0-mL analysis volume is taken. A pH adjustment is necessary before analysis.

Use equation 2: Equation 2 may be used when a minimum sample size is necessary or when a sample has already been digested for one parameter (such as copper) and measurement for another parameter (such as zinc) is necessary. Continue the example for copper, above, a zinc test may also be done. The undigested sample contains approximately 3 ppm zinc and the Zincon method is used. The analysis volume can be found as follows.

In this example, the Zincon method test range is thought to be 0–2.5 ppm so that the midpoint of the range is 1.25. Therefore values are:

A = 3

B =1.25

C = 100

D = 50

E = 8 (as found above)

substitute: F = (1.25 × 100 × 50) ÷ (3 × 8) = 260 mL

This is an extreme example, but it shows the need to compare the values of D and F to make sure that the analysis volume (F) is no more than the final analysis volume (D). If F exceeds D, the analysis cannot be done. A test with a more applicable range is necessary or a larger sample may be digested for this test. Care must also be taken to make sure that the volume of digest taken for analysis (F) is higher than 0.1 mL because accurately pipetting less than 0.1 mL is difficult.

As a comparison, think of the zinc concentration as 75 ppm (A = 75 instead of 3) and substitute again to get:

F = (1.25 × 100 × 50) ÷ (75 × 8) = 10.5 mL

In this case, the aliquot volume is less than the final analysis volume so analysis may be done as specified in the procedure.

Why is the factor in the calculation step 75, 2500 or 5000 (depends on the method used) and where does the factor come from?

In all cases, the factor is a correction for sample dilution. For example, in some tests the factor is 2500. The Digesdahl digestion total volume is 100 mL, the analysis total volume is 25 mL and 100 x 25 = 2500. The mL units are not included with the factor because they cancel out in the formula.

When a slurry is analyzed, how is the total concentration on a dry basis reported?

The sample must be analyzed for moisture content. For necessary apparatus, refer to Table 14 and Table 15.

To find the dry basis weight:

1. Weigh an aluminum dish and record the weight as “A”.
2. Weigh out approximately 2 g of solid sample into the dish. Record the exact weight added as “B.”
3. Put the dish in the oven (103–105 °C, 217–221 °F) for 2 hours.
4. Put in a desiccator and cool to room temperature.
5. Weigh the aluminum dish with the oven-dried sample. Record as “C.”
   Note: *The oven-dried material generally is not meant for additional testing and should be discarded.*
6. Use this formula to calculate the sample on a “dry basis.” Test result (dry basis) = (C – A) ÷ (B – A).
   Note: *Multiply the test result on an “as is” basis, by the factor above, to report as “dry basis".*

Table 14 Necessary apparatus for dry basis weight

Description	Unit	Item no.
Balance, analytical, 120-g	454 g	2936801
Desiccant, Drierite (without indicator)	each	2285901
Desiccator, vacuum (uses ceramic plate)	100/pkg	2088800
Dish, moisture determination, aluminum, 63 x 17.5 mm	each	2164000
Tongs, crucible	each	56900
Oven, laboratory, 120 VAC	each	1428900
or		
Oven, laboratory, 240 VAC	each	1428902

Table 15 Optional apparatus

Description	Unit	Item no.
Desiccator, without stopcock	each	1428500

5.2.2 Adjust the pH

5.2.2.1 For a metals procedure

Note: *If aliquots smaller than 0.5 mL are analyzed, pH adjustment is not necessary.*

1. Find the necessary volume of sample for analysis from the Sample and Analysis Volume Tables after each digestion procedure. Use a pipet to add this volume into a graduated mixing cylinder.
   Note: *To use a pipet to add a volume into a volumetric flask or a regular graduated cylinder is necessary for some methods.*
2. Dilute to about 20 mL with deionized water.
3. Add one drop of 2,4 Dinitrophenol Indicator Solution.
4. Add one drop of 8 N Potassium Hydroxide (KOH) Standard Solution (28232H). Swirl after each addition until the first flash of yellow shows (pH 3). If the sample is analyzed for potassium, use 5 N sodium hydroxide (245026) instead. Do not use a pH meter if the sample is analyzed for potassium or silver.
5. Add one drop of 1 N KOH (2314426). Put the stopper in the cylinder and invert several times to mix. If the sample is analyzed for potassium, use 1 N sodium hydroxide instead.
   Note: *Use pH paper to make sure that the pH is 3. If it is higher than 4, do not adjust again with acid. Start over with a fresh aliquot.*
6. Continue to add 1 N KOH in this manner until the first permanent yellow color shows (pH 3.5–4.0).

7. Look at the cylinder from the top against a white background. Compare the cylinder to a second cylinder filled to the same volume with deionized water.
   Note: *High iron content will cause precipitation (brown cloud) which will co-precipitate other metals. Do this procedure again with a smaller aliquot volume.*
8. Add deionized water to the volume specified in the colorimetric procedure for the parameter under analysis.
9. Continue with the colorimetric procedure.

5.2.2.2 For the Total Kjeldahl Nitrogen colorimetric method

Consult the spectrophotometer or colorimeter procedure to complete the TKN analysis. The procedure that follows is only a guide to use if a procedure is not available.

1. Use a pipet to add an appropriate analysis volume to a graduated mixing cylinder.
2. Add one drop of TKN Indicator (2251900).
3. Add one drop of 8 N KOH Standard Solution (28232H), swirl after each addition until the first flash of pale blue shows (pH 3).
4. Add one drop of 1 N KOH (2314426). Put the stopper in the cylinder and invert several times to mix.
   Note: *Look at the cylinder from the top against a white background. Compare the cylinder to a second cylinder filled to the same volume with deionized water.*
5. Continue to add 1 N KOH in this manner until the first permanent blue color shows.
6. Add deionized water to the volume shown in the colorimetric procedure for the parameter under analysis.
7. Continue with the colorimetric procedure.

Section 6 Bacteria analysis

The amount and type of bacteria in water samples is routinely measured to find whether the water contains disease-causing organisms. All tests for bacteria use a nutritional broth or agar and incubation at a specific temperature to grow the target organism. Sterile equipment and careful handling techniques are necessary to prevent contamination of the sample.

6.1 About indicator organisms

Bacterial pathogens that cause serious diseases are difficult to detect in water supplies and include long and complex test procedures. Thus, tests for indicator organisms that have a fecal origin such as coliform bacteria are commonly used. Indicator organisms may not be pathogenic but are present when pathogens are present and absent when pathogens are absent.

Total coliform bacteria are commonly used as indicator organisms in potable water supplies in temperate climates. Fecal coliform bacteria, and more specifically Escherichia coli, are commonly used as indicator organisms for non-potable water, wastewater, bathing water and swimming water.

In tropical climates, indigenous *Escherichia coli (E. coli)* bacteria give positive results in total coliform tests even in pristine water sources where no fecal contamination exists. Thus, other bacteria that are associated with fecal contamination such as hydrogen sulfide-producing bacteria are used as an indicator organism.

6.2 Test methods for bacteria

Test methods for bacteria show whether or not an indicator organism is in a water sample. Some methods count or make an estimate of the number of organisms in the water. Refer to Table 16.

Table 16 Bacteria test methods

Method name	Method description
Presence/Absence (P/A)	The sample is mixed with a nutritional broth that contains a color indicator and incubated. A color change is an indication that the target bacteria may be in the sample.
Most Probable Number (MPN)	The sample is diluted and added to a series of tubes that contain a nutritional broth. After incubation, the tubes are examined for the presence of gas.
Membrane Filtration (MF)	The sample is filtered and the filter is put on nutritional media in a petri dish. After incubation, the filter is examined for bacteria colonies.
Plate count agar	The sample is mixed with a nutritional agar in a large petri dish and incubated. After incubation, the agar is examined for bacteria colonies. This method is typically used for total or heterotrophic bacteria.

6.3 Presumptive and confirmation procedures

Most test methods for bacteria begin with a presumptive test procedure. If the result from the presumptive test is positive, a confirmation procedure must be completed. Some media is selective for the target organism and a confirmation test is not required. For example, the m-ColiBlue24® broth and broth that contains MUG is selective for *E. coli*.

- Presumptive test—a positive result is an indication of the target organism but can include a false positive result.
- Confirmation test—the cultured bacteria from the presumptive test are used to inoculate the confirmation media. The confirmation media is more selective for the target organism and may use a higher incubation temperature.

6.4 Preparation for bacteria tests

Good laboratory technique is necessary for bacteria tests. To make sure that the results are reliable, collect and preserve samples carefully. Use high-quality laboratory equipment and ready-to-use media to save time and prevent errors.

6.4.1 Prepare the work area

- Wash hands thoroughly with soap and water.
- Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. A small propane torch can be used to flame-sterilize metal faucets that are used for sample collection.
- Set the incubator to the temperature that is specified by the test procedure. Typically 35 ± 0.5 °C (95 ± 0.9 °F) is used for total coliforms and enterococci and 44.5 ± 0.2 °C (112.1 ± 0.4 °F) is used for fecal coliforms.

6.4.2 Sterilize laboratory equipment

All materials that are used to contain or transfer samples must be sterile to prevent contamination and false results. Use pre-sterilized, disposable laboratory equipment and media to save time and minimize errors. When numerous analyses must be completed on a routine basis, sterilization of non-disposable materials with an autoclave is recommended.

1. Wash sample containers and any necessary equipment with hot water and detergent. Some procedures use equipment such as pipets, petri dishes, a filter holder with stopper and a graduated cylinder.
2. Rinse containers and equipment several times with tap water and then with deionized water.
3. If the water to be sampled has been disinfected by some entity before collection, add the contents of one Dechlorinating Reagent Powder Pillow for each 125 mL of container volume (for 250-mL sample containers, use two powder pillows.)
4. Prepare all equipment for the autoclave as follows:
   a. Loosely install the caps on sample bottles and put foil or paper on caps and bottle necks.
   b. Put foil or paper over the openings of graduated cylinders.
   c. Insert the filter funnel base into an autoclavable rubber stopper that will fit the filter flask.
   d. Put heavy wrapping paper around the two parts of the filter funnel assembly and seal with masking tape.
   e. Put paper around petri dishes (borosilicate glass) or put in aluminum or stainless steel cans.
5. Put the containers and equipment in the autoclave. Steam sterilize the containers and equipment at 121 °C (250 °F) for 15 minutes. Glass sample containers can be sterilized with hot air at 170 °C (338° F) for 1 hour.
6. When sterilization is complete, put on sterile gloves and tighten the caps on the containers and equipment. Put the labware in a clean environment until needed.

6.5 Sample collection and preservation

Collect a sufficient volume of sample for analysis (usually a minimum of 100 mL of sample). The World Health Organization guidelines prescribe 200 mL per sample. Standard Methods for the Examination of Water and Wastewater prescribes 100 mL per sample. Prevent sample contamination.

No dechlorination is necessary if the sample is added directly to the growth medium on site. Otherwise, add a dechlorinating reagent to the samples to remove the chlorine residual. Sodium thiosulfate that has been sterilized within the collection vessel is used to remove chlorine residual. Transport for analysis immediately after collection.

Analyze the samples as soon as possible after collection. If the analysis cannot be started immediately, keep the sample at or below 10 °C (50 °F), but do not freeze. The maximum time between sample collection and incubation is 8 hours. Failure to collect and transport samples as specified will cause inaccurate results. Refer to the local regulatory agency for the most current holding times and temperatures.

Use sterilized plastic bags or disposable bottles

Use presterilized Whirl-Pak® bags or bottles for sample collection. If the sample has been disinfected, use bags or bottles that contain a dechlorinating agent. Bags or bottles that contain dechlorinating reagent can be used for all samples because the dechlorinating reagent does not interfere with untreated samples. Autoclavable glass or plastic bottles can also be used.

Write the sample number, dilution, date and other necessary information on each sample container. Use aseptic technique to prevent contamination of the inside of the sample container.

Faucets, spigots, hydrants or pumps

1. Let the water flow at a moderate rate for 2 to 3 minutes (potable water).
2. Adjust the flow before the sample collection to prevent spills and splashes. Do not adjust the flow during the sample collection. Do not use valves, spigots and faucets that swivel or leak. Remove any aerators or screens.
3. Collect a minimum of 100 mL of the sample in a sterilized container. Do not fill the sample containers completely. Keep a minimum of 2.5 cm (1 inch) of air space to help mix the sample before analysis.
   Note: *Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inside surface of the container. Do not rinse the containers before use.*
4. Write the sample information on the container and start the analysis as soon as possible.

Rivers, lakes and reservoirs

1. Do not collect samples near the edge of the river, lake or reservoir.
2. If possible, remove the cap under water. As an alternative, remove the cap, grasp the sample container near the bottom and plunge the container, mouth down, into the water to exclude any surface scum.
3. Fill the container entirely under water. Put the mouth of the container into the current or, in non-flowing water, tilt the container slightly and let the container fill slowly. Do not rinse the container before use.
4. Write the sample information on the container and start the analysis as soon as possible.

6.6 Dilution for non-potable samples

Non-potable water samples must be diluted to a level at which the bacteria can be measured.

1. Wash hands.
2. Open a 99-mL bottle of sterile Buffered Dilution Water.
3. Shake the sample container in a waist to ear motion, approximately 25 times for 30 seconds.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.

5. Put the cap on the dilution water bottle and invert the sample container in a waist to ear motion 25 times for 30 seconds. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions to the level that is specified for the test procedure.

6.7 Dispose of bacteria cultures

To safely dispose of bacterial cultures after completed tests, use one of the methods that follow.

- **Bleach**—Sterilize the used test containers with household bleach. Add 1–2 mL of the bleach to each test tube. Keep 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.
- **Autoclave**—Put the used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C (250 °F) for 30 minutes at 15 pounds pressure. When cool, seal the bag, put it in another garbage bag and use a tie to close the bag tightly.

Section 7 Waste management and safety

This section provides guidelines for laboratory waste management. These guidelines are only a summary of basic USEPA requirements and do not relieve the user from compliance with the complete regulations contained in the Code of Federal Regulations (CFR). The regulations may change or additional state and local laws may apply. Waste generators are responsible for knowing and obeying all the laws and regulations that apply to their operations.

7.1 Waste minimization

The most effective way to decrease waste management problems and expense is through waste minimization. To do this:

- Use the smallest sample size that will make accurate results.
- Where possible, do methods that use reagents that pose fewer hazards.
- Purchase in smaller quantities to remove the need to dispose of out-dated materials.
- Use biodegradable detergents to clean glassware and apparatus unless solvents or acids are a specific requirement.

7.2 Regulatory overview

The Resource Conservation and Recovery Act (RCRA) controls all solid waste disposal with an emphasis on hazardous waste. Title 40 Code of Federal Regulations (CFR) part 260 contains the federal hazardous waste disposal regulations issued in accordance with the RCRA. The regulations create a system to identify hazardous wastes and track waste generation, transport and ultimate disposal from beginning to end. Each facility involved in hazardous waste management must be registered with the USEPA, with the exception of conditionally exempt small quantity generators.

Federal regulations recognize three categories of generators and those who make larger amounts of waste are under stricter control. The categories are:

- Conditionally exempt small quantity generator—less than 100 kg (220 lb) per month
- Small quantity generator—between 100 kg (220 lb) and 1000 kg (2200 lb) per month
- Large quantity generator—higher than 1000 kg (2200 lb) per month

7.3 Hazardous waste

7.3.1 Definition

For regulatory purposes, a hazardous waste is a material that is subject to special consideration by the USEPA under 40 CFR 261. State or local authorities may also designate additional materials as hazardous waste in their areas.

Many toxic compounds are not regulated, but improper management or disposal may lead to legal problems under CERCLA (Superfund) or common law tort.

The definition given by 40 CFR 261 identifies a hazardous waste as a solid waste that is not excluded from regulation and meets one or more of the criteria that follow:

- It is a discarded commercial chemical product, off-specification species, container residue or spill residue of materials specifically listed in 40 CFR 261.33 (P- and U-codes)
- It is a waste from a specific source listed in 40 CFR 261.32 (K-code)
- It is a waste from a non-specific source listed in 40 CFR 261.31 (F-code), and/or
- It shows any of the characteristics of hazardous waste that follow:
    - Ignitability
    - Corrosivity
    - Reactivity

- Toxicity

There are exceptions to these regulations. Look at the regulations to find applicable exclusions.

7.3.2 Sample codes

Hazardous wastes are managed by specific codes assigned in 40 CFR 261.20–261.33. These codes are provided to help identify hazardous waste. The generator is responsible to make the actual waste code determination.

Selected characteristic waste codes for chemicals which may be generated with methods for water analysis are given in Table 17. A complete list of waste codes is found in 40 CFR 261.20 through 40 CFR 261.33.

Table 17 Hazardous waste codes

Characteristic	USEPA code	Chemical abstract services (CAS) number	Regulatory level (mg/L)
Corrosivity	D002	—	—
Ignitability	D001	—	—
Reactivity	D003	—	—
Arsenic	D004	6440-38-2	5.0
Barium	D005	6440-39-3	100.0
Benzene	D018	71-43-2	0.5
Cadmium	D006	7440-43-9	1.0
Chloroform	D022	67-66-3	6.0
Chromium	D007	7440-47-3	5.0
Lead	D008	7439-92-1	5.0
Mercury	D009	7439-97-6	0.2
Selenium	D010	7782-49-2	1.0
Silver	D011	7440-22-4	5.0

7.3.3 How to tell if waste is hazardous

Federal laws do not require material testing to find out if waste is hazardous. If the product is not specifically shown in the regulations, apply product or generator knowledge to find out if it is hazardous. Often, there is enough information on a Safety Data Sheet (SDS) to decide. Look for characteristics of a hazardous waste:

- The flash point is below 60 °C (140 °F) or it is classified by DOT as an oxidizer (D001).
- The pH of the material is less than or equal to pH 2, or higher than or equal to 12.5 (D002).
- The material is unstable, reacts violently with water, may make toxic gases when mixed with water (D003).
- It is toxic (D004–D043).

Use the chemical composition data to find out if a material is toxic based on the concentration of certain contaminants (heavy metals and a number of organic compounds). If the waste is a liquid, compare the concentration of contaminants to the concentrations shown in 40 CFR 26. If the waste is a solid, make an analysis of the sample with the Toxicity Characteristic Leachability Procedure (TCLP) and then compare the results to the concentrations in 40 CFR 261.24. Levels above the threshold amounts should be thought of as hazardous.

For more information about SDS usage, refer to Safety data sheets on page 56.

Some tests use or make a number of chemicals that make the end product a hazardous waste (e.g., the COD tests and Nessler reagent). Hazardous waste status may also result from substances present in the sample.

7.3.4 Disposal

Hazardous waste must be managed and discarded according to federal, state and local regulations. The waste generator is responsible for hazardous waste determinations. Analysts should speak with their facility environmental compliance department for specific instructions.
Most hazardous wastes should be moved by treatment, storage and disposal facilities (TSDF) that have USEPA permits. In some cases, the generator may treat the hazardous waste, but may need a permit from the USEPA and/or state agency. Laboratories are not exempt from these regulations. If the facility is a “Conditionally Exempt Small Quantity Generator,” special rules may apply. Look at 40 CFR 261 to find the laws and rules that apply for a given generator.

The most common acceptable treatment is elementary neutralization. This applies to wastes that are hazardous only because they are corrosive or are listed only for that reason. Many generated wastes may be treated with the steps that follow:

1. To neutralize acidic solutions, add a base such as sodium hydroxide. To neutralize basic solutions, add an acid such as hydrochloric acid.
2. Slowly add the neutralizing agent while the solution is stirred.
3. Monitor the pH.
4. When the pH is at or near pH 7, the material is neutralized and may be flushed down the drain.

For other chemical or physical treatments, such as cyanide destruction or evaporation, a permit may be necessary. Speak with the environmental department or local regulators to find which rules apply to each facility.

Laboratory chemicals may be mixed and disposed of with other hazardous wastes generated at a facility. They may also be collected in accordance with 40 CFR 262.34 satellite accumulation rules. After collection, they may be disposed of in a labpack. Many environmental and hazardous waste companies offer labpacking services. These companies will inventory, sort, pack and arrange for proper disposal of hazardous waste. Find companies that offer these services in the telephone book under “Waste Disposal—Hazardous” or contact state and local regulators for assistance.

7.4 Management of specific waste

Recycling programs for some forms of laboratory waste are available through Hach Company. To get information on recycling services, call 1-800-227-4224 or visit www.hach.com.

Several documents are also available to assist in the management of generated waste. To get the documents, call 1-800-227-4224 or 970-669-3050 and request the literature codes in Table 18.

Table 18 Waste management literature

Literature code	Title
9323	Mercury Waste Disposal Firms
9324	RCRA Waste Disposal Information
9325	COD Waste Disposal Information
9326	COD Heavy Metal Concentrations

7.4.1 Special considerations for Cyanide-containing materials

Several procedures in this manual use reagents that contain cyanide compounds. These materials are regulated as reactive waste (D003) by the Federal RCRA. Instructions provided with each procedure tell how to collect these materials for proper disposal. It is imperative that these materials be moved safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of bitter almonds). Most cyanide compounds are stable and can be safely kept for disposal, in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes that may contain lower pH materials such as acids or even water.
If a cyanide-containing compound is spilled, avoid exposure to hydrogen cyanide gas. Do the steps that follow to destroy the cyanide compounds in an emergency:

1. Use a fume hood, supplied air or self-contained breathing apparatus.
2. Stir as the waste is added to a beaker that contains a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).
3. Add an excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
4. Neutralize the solution and flush it down the drain with a large amount of water. If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Do not flush untreated hazardous wastes down the drain.

7.5 Resources

Many sources of information on proper waste management are available. The USEPA has a hotline number for questions about the Resource Conservation and Recovery Act (RCRA). The RCRA Hotline number is 1-800-424-9346. Copies of the applicable regulations are available. Federal hazardous waste regulations are found in 40 CFR 260-99. Get this book from the U.S. Government Printing Office or an alternate vendor. Other documents that may be helpful to the hazardous waste manager in the laboratory include:

- Task Force on Laboratory Waste Management. Laboratory Waste Management, A Guidebook; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1994.
- Task Force on Laboratory Waste Management. Waste Management Manual for Laboratory Personnel; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1990.
- Task Force on Laboratory Waste Management. Less is Better; 2nd ed.; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1993.
- Committee on Chemical Safety. Safety in Academic Chemistry Laboratories, 5th ed.; American Chemical Society: Washington, DC, 1990.
- Armour, Margaret-Ann. Hazardous Laboratory Chemicals Disposal Guide; CRC Press: Boca Raton, FL, 1991.
- Environmental Health and Safety Manager’s Handbook; Government Institutes, Inc.: Rockville, MD, 1988.
- Lunn, G.; Sansone, E.B. Destruction of Hazardous Chemicals in the Laboratory; John Wiley and Sons: New York, 1990.
- National Research Council. Prudent Practices for Disposal of Chemicals from Laboratories; National Academy Press: Washington, DC, 1983.
- National Research Council. Prudent Practices for Handling Hazardous Chemicals in Laboratories; National Academy Press: Washington, DC, 1981.
- Environmental Protection Agency, Office of Solid Waste and Emergency Response. The RCRA Orientation Manual; U.S. Government Printing Office: Washington, DC, 1991.

- Environmental Protection Agency, Office of Solid Waste and Emergency Response. Understanding the Small Quantity Generator Hazardous Waste Rules: A Handbook for Small Business; U.S. Government Printing Office: Washington, DC, 1986.

7.6 Safety

Safety is the responsibility of every analyst. Many chemical procedures use potentially hazardous chemicals and equipment. It is important to use good laboratory techniques and prevent accidents. The guidelines that follow apply to water analysis and are not intended to cover every aspect of safety.

7.6.1 Read labels carefully

Read each reagent label carefully. Note the precautions given. Do not remove or cover the label on a container while it contains reagent. If a different reagent is put into a labeled container, the label must be changed. When a reagent or standard solution is prepared, label the container clearly. If a label is hard to read, replace the label promptly according to the hazard communication program.
Warning labels also show on some of the apparatus used with the test procedures. The protective shields with the Digesdahl Digestion Apparatus point out potential hazards. Make sure that these shields are installed during use and obey the precautions on the label.

7.6.2 Protective equipment

Use the applicable protective equipment for the chemicals and procedures. The SDS contains this information. Protective equipment may include:

- Eye protection, such as safety glasses or goggles, to protect from flying objects or chemical splashes.
- Gloves to protect skin from toxic or corrosive materials, sharp objects, very hot or very cold materials or broken glass. Use tongs or finger cots when a hot apparatus is transferred.
- Laboratory coats or splash aprons to protect skin and clothing from splashes.
- Footwear to protect feet from spills. Open toed shoes should not be worn in chemistry settings.
- Respirators may be necessary if sufficient ventilation, such as fume hoods, are not available. Use fume hoods when directed to do so by the procedure or as recommended in the SDS. For many procedures, adequate ventilation is enough if there is plenty of fresh air and air exhaust to protect against unnecessary exposure to chemicals.

7.6.3 First aid equipment and supplies

Most first aid instructions for chemical splashes in eyes or on skin call for a thorough flush with water. Laboratories should have eyewash and shower stations. For field work, carry a portable eyewash unit. Laboratories should also have the necessary fire extinguishers and fume hoods.

7.6.4 General safety rules

Obey these rules when work is done with toxic and hazardous chemicals:

- Never pipet by mouth. Always use a mechanical pipet or pipet bulb to avoid ingestion of chemicals.
- Follow test procedures carefully and observe all precautionary measures. Read the entire procedure before the procedure is started.
- Clean all spills promptly. Get proper training and have the right response equipment to clean up spills. Refer to the safety director for more information.
- Do not smoke, eat or drink in an area where toxic or irritating chemicals are used.
- Use reagents and equipment only as directed in the test procedure.
- Do not use damaged labware and broken equipment.

- Minimize all chemical exposures. Do not breathe vapors or let chemicals touch the skin. Wash hands after chemicals are used.
- Keep work areas neat and clean.
- Do not block exits or access to emergency equipment.

7.7 Safety data sheets

Safety data sheets (SDS) describe the hazards of chemical products. This section describes the information found on the SDS and tells how to find important information for safety and waste disposal. The information provided on the SDS applies to the product as sold by a specific manufacturer. The properties of any mixtures made with this product will be different.

7.7.1 How to get an SDS

The SDS is shipped to a customer with the first order of any chemical product. A new SDS may be sent when the information on the data sheet is updated. Review all new SDS documents for new information. To get another copy of an SDS, call 1-800-227-4224 or download it directly from www.hach.com.

7.7.2 Sections of an SDS

Each SDS has 10 sections. The sections and the information included are described below.

Header information

The manufacturer order number, SDS date, change number, company address and telephone number and emergency telephone numbers are shown at the top of the SDS.

7.7.2.1 Product identification

This sections contains:

- Product name
- Chemical Abstract Services (CAS) number
- Chemical name
- Chemical formula, if applicable
- Chemical family to which the material belongs

7.7.2.2 Ingredients

This section shows each component in the product. It contains the information that follows for each component:

- PCT: Percent by weight of this component
- CAS NO.: Chemical Abstract Services (CAS) registry number for this component
- SARA: Superfund Amendments and Reauthorization Act, better known as the “Community Right to Know Law,” tells if the component is shown in SARA 313. If the component is shown and the facility uses more than the specified amount, report use to the USEPA every year.
- TLV: Threshold Limit Value. The maximum airborne concentration for an 8-hour exposure that is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).
- PEL: Permissible Exposure Limit. The maximum airborne concentration for an 8-hour exposure that is regulated by the Occupational Safety and Health Administration (OSHA).
- HAZARD: Physical and health hazards of the component are explained.

7.7.2.3 Physical data

The physical properties of the product are given in this section. The physical properties include the physical state, color, odor, solubility, boiling point, melting point, specific gravity, pH, vapor density, evaporation rate, corrosivity, stability and storage precautions.

7.7.2.4 Fire and explosion hazard and reactivity data

This section contains the flash point and flammable limits of the material. It also includes how to fight fires if the material catches on fire. Key terms in this section include:

- Flash point: The temperature at which a liquid will give off enough flammable vapor to ignite.
- Flammability and ignitability are usually defined by the flash point.
- Lower Flammable Limit (LFL or LEL): The lowest concentration that will cause a fire or flash when an ignition source is present.
- Upper Flammable Limit (UFL or UEL): The vapor concentration in air that has a concentration that is too rich to burn.
- NFPA Codes: The National Fire Protection Association (NFPA) has a system to rate the degree of hazards given by a chemical. These codes are usually put in a colored diamond. The codes range from 0 for minimal hazard to 4 for extreme hazard. The codes are grouped into the hazards that follow: health (blue), flammability (red), reactivity (yellow) and special hazards (white).

7.7.2.5 Health hazard data

This section describes the pathways for a chemical to enter the body (i.e., ingestion, inhalation, skin contact). It also gives acute (immediate) and chronic (long-term) health effects. If the material causes cancer or genetic damage, it is specified in this section.

7.7.2.6 Precautionary measures

This section contains special precautions for the material. The precautions may include special storage instructions, handling instructions, conditions to avoid and protective equipment necessary to use this material safely.

7.7.2.7 First aid

First aid instructions for exposures to the chemical are given in this section. Be sure to read this section before a victim is induced to vomit. Some chemicals are better treated if the victim does not vomit. Get immediate medical attention for all chemical exposures.

7.7.2.8 Spill and disposal procedures

This section describes safe practices for the clean up and disposal of spilled material. For more information, refer to Hazardous waste on page 51. The waste generator is ultimately responsible to meet the federal, state and local laws that apply to each facility.

7.7.2.9 Transportation data

Domestic and international shipping information is provided in this section. The shipping name, hazard class and ID number of the product are given.

7.7.2.10 References

This section shows the reference materials used to write the SDS.

Refer to the Reference section that shows that this product has SARA 313 chemicals or California Proposition 65 List Chemicals, if applicable. Any special information about the product is found here.

7.7.3 OSHA chemical hygiene plan

The Occupational Safety and Health Administration (OSHA) enforces laws controlling exposure to hazardous chemicals in laboratories. These regulations are found in Title 29 CFR 1910.1450. The regulations apply to all employers who use hazardous chemicals and make it necessary for employers to develop and use a written Chemical Hygiene Plan and to appoint a qualified person as the Chemical Hygiene Officer.

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