

An Introduction to Standards and Quality Control for the Laboratory

Barbara Martin

In memory of
Clifford C. Hach
(1919-1990)

inventor, mentor, leader and, foremost,
dedicated chemist

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In a laboratory or plant, there are many situations when the accuracy of a laboratory analysis must be proven. Important decisions are based on the results of chemical testing. It is vital that the results of chemical analyses are correct. This book focuses on the use of standard solutions as a verification tool for colorimetric analysis. Standard solutions are used to calibrate instruments used for colorimetric, electrochemical, and turbidimetric measurements. Standards are also useful for verifying just about any method of analysis including electrochemical, titrametric, turbidimetric, and other analytical methods.

1.1 Standard Solutions

A standard solution is a solution that contains a known concentration of a particular chemical, or analyte. Standard solutions can be used in many different ways, with one commonality—they can be used to establish the accuracy and precision of a test method.

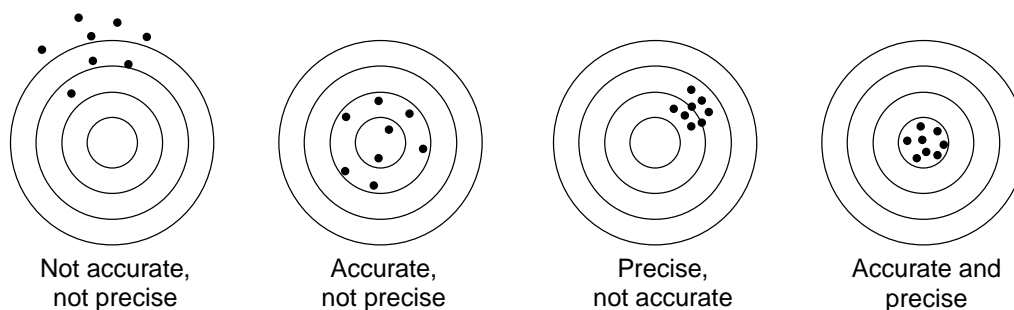
Accuracy is a measure of the nearness of an analytical result to the true value. In order for accuracy to be assessed, the true concentration of a solution must be known. While the true concentration of an analyte in a sample is never known, a standard solution has a known concentration of analyte and can be used to assess accuracy.

Precision determines the consistency, or repeatability, of analytical measurements. Testing standard solutions may aid in the improvement of precision and accuracy of chemical analyses. Refer to Figure 1 for examples of accuracy and precision.

Standard solutions are available for many different analytes (and in many concentrations) including nitrate, fluoride, hardness, phosphate, ammonia, and most metals. A standard solution or solid standard (from which a solution can be prepared) is available for many parameters corresponding with Hach tests.

Although many analysts test a sample stream with a consistent chemical composition, a sample analysis cannot be used to verify performance because the exact concentration of a species in a sample is not known. When testing a sample, the analyst is unable to know if the results of the test match the true concentration in the sample. However, when using a standard solution, the analyst knows the true concentration and is able to verify the test results.

Figure 1 Precision and Accuracy Illustrated



Introduction to Standards

For example, if an analyst tests a 1.00 mg/L fluoride standard solution, they should find their fluoride measurements to be very close to 1.00 mg/L, typically within 10%. This allows the analyst the ability to verify that they are running the test correctly and reading accurate results for the parameter.

1.2 Using Standards to Help with Testing

Accurate measurement of a standard can help ensure that the results of a chemical analysis are correct. If standard measurements are not used, inconsistent results leave the analyst wondering about the accuracy of the instrument, reagent, and technique.

Knowing that standard results are correct can help in many ways:

- Saves time and money—The entire test system is verified before spending time and reagents testing actual samples.
- Assesses the skills and techniques of the employee or analyst.
- Prevents scrap product or excess chemical usage due to a faulty test system.
- Proves results to inspectors or customers.
- Reduces amount of time spent troubleshooting problems with chemical testing. May reveal problems, to an analyst, before they are detected or cause a larger problem in the treatment system.
- Verifies the performance between two instruments, for example, a laboratory spectrophotometer with an online CL17 Chlorine Analyzer. If readings from a CL17 chlorine analyzer and laboratory instrument do not match, the analyst should run a standard on the laboratory instrument. If inaccurate results are obtained, further troubleshooting is required to assess the accuracy of the laboratory instrument. Running a standard solution does not completely solve the problem, but it narrows down the troubleshooting options in an otherwise complicated situation.

1.3 Using Standards

Many Hach standard solutions are at suitable concentrations and can be used directly out of the bottle. In this case, the standard is poured into a sample cell and tested the same way as a typical sample. For example, Hach offers the FerroVer method for iron, which has an analysis range of 0–3.00 mg/L. A 1.00 mg/L iron standard can be run directly out of the bottle for this test because the concentration lies within the test's measurement range.

Some standard solutions have a concentration outside the analysis range of a test. For example, Hach offers a 25–30mg/L chlorine standard. This standard cannot be tested directly using the 0–2.00mg/L DPD chlorine chemistry. The standard must be diluted to a level within the method's measurement range before it is tested.

Many concentrated Hach standards are available in 2-mL or 10-mL sealed glass ampules. The volumes in these ampules are approximate, since each ampule contains slightly more volume than 2 or 10mL. When preparing a standard solution from one of these ampules, be sure to use a pipet for accurate volumetric measurement.

Note: Use the standard recommended in the procedure whenever possible. Be careful when using standards from another manufacturer. Sometimes these standards are intended for another type of analysis and may contain preservatives or extreme pH values that may interfere with the analysis method.

1.3.1 Preparing Dilutions

Dilutions must be prepared using high quality deionized water. Deionized water (also known as demineralized) is water from which all ions have been removed. Such clean water is required to prepare dilutions so that the concentration of the diluted standard can be known exactly. **Dilutions cannot be prepared using tap water or sample water.** Problems arise if the water used for a dilution is not of the highest quality—especially if it contains additional analyte or interferences. If a lab does not have a source of high quality water, deionized water can be purchased from Hach.

Diluted standards are prepared from concentrated standard solutions. Although concentrated standard solutions are typically stable for many months, most diluted standards should not be considered stable. Diluted standards and samples should be used immediately after preparation and discarded after use. Storage and re-use of diluted standard or sample is not recommended. For best results, follow the manufacturer's instructions regarding the stability of dilutions.

An accurate dilution is easy to prepare. Refer to Figure 2 and the following steps:

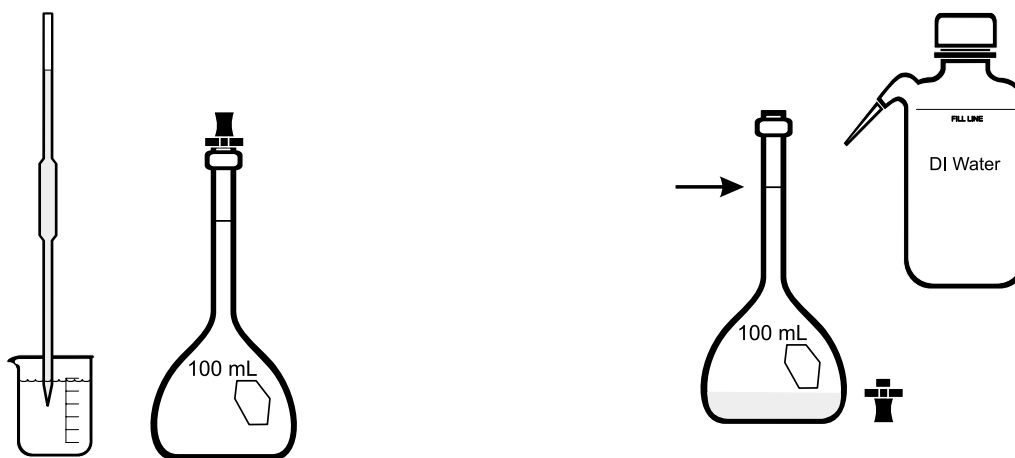
1. Use a volumetric or automatic pipet to dispense the chosen volume of concentrated standard into a clean volumetric flask.

Note: A graduated cylinder can be used if volumetric glassware is not available. However, standards prepared in a graduated cylinder will not be as accurate as those prepared using volumetric glassware.

2. Fill the flask to volume with deionized water. Fill the flask with water until the bottom of the meniscus rests on the top of the volumetric mark that is either etched or painted on the flask.
3. Invert the flask several times to thoroughly mix the solution. Once the dilution is prepared, the test can be run on the diluted standard.

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Figure 2 Preparing a Dilution



Pipet the chosen volume of standard into a clean volumetric flask.

Dilute to the mark with deionized water. Stopper and invert several times to mix.

Case 1–A 100-mL Final Volume

Dilution reduces the concentration of the original standard by a certain amount. This amount depends on how the dilution is performed. Suppose a 200.0-mg/L iron standard is diluted by dispensing 1.00 mL of standard into a 100-mL volumetric flask and filling it to the line with deionized water. Taking the original standard concentration and dividing by the factor in the far right column of Table 1 calculates the final concentration of the standard:

$$200 \text{ mg/L} / 100 \text{ (dilution factor from table)} = 2.00 \text{ mg/L}$$

The diluted standard has a concentration of 2.00 mg/L. If an iron test is run on this standard, the reading should be approximately 2.00 mg/L.

Table 1 Division Factors for Various 100-mL Dilutions*

Standard Volume (mL)	mL Deionized Water to Bring Total Volume to 100.0 mL	Divide Original Concentration by this Factor
1.0	99.0	100
2.0	98.0	50
5.0	95.0	20
10.0	90.0	10
20.0	80.0	5
25.0	75.0	4
50.0	50.0	2

* The factors listed in this table are only accurate for dilutions prepared with a 100 mL final volume. To calculate the concentration of a 100 mL dilution, divide the original standard concentration by the factor listed in the right hand column.

Case 2—Other Volumes

It may be necessary to dilute standards to a final volume other than 100 mL. In this case, Table 1 cannot be used to calculate the final standard concentration. When other volumes are used, the formula below can be used to calculate either the final standard concentration or the volume of a standard required to prepare the dilution:

$$C_1V_1 = C_2V_2$$

Where:

C_1 = Original standard concentration (obtained from label or Certificate of Analysis)

V_1 = Volume of standard required to prepare the dilution

C_2 = Diluted standard concentration

V_2 = Total dilution volume

This equation can be used in two ways—calculating the concentration of a diluted standard or calculating the volume of a concentrated standard required to prepare a certain dilution.

Note: There can only be one unknown when solving the equation. Therefore, the equation can be used to solve for only one factor. Examples of each are described below.

Example 1: Calculating the concentration of a diluted standard

A 100-mg/L fluoride standard is diluted by dispensing 10.0 mL of standard into a 1000.0-mL volumetric flask and filling it to the line with deionized water. To calculate the concentration of the diluted standard, use the equation below and substitute the known values:

$$C_1V_1 = C_2V_2$$

$$(100 \text{ mg/L})(10.0 \text{ mL}) = C_2(1000.0 \text{ mL})$$

$$C_2 = 1.00 \text{ mg/L}$$

Therefore, if a fluoride test is run on this standard, the reading should be approximately 1.00 mg/L.

Example 2: Calculating the volume of a standard required for dilution

A 10.0-mg/L nitrate standard is required for testing. The lab has a 50.0-mg/L nitrate standard, some pipets, and a 100.0-mL volumetric flask. To calculate the volume of 50.0-mg/L standard required to prepare 100.0 mL of a 10.0-mg/L standard, use the equation below and substitute the known values:

$$C_1V_1 = C_2V_2$$

$$(50.0 \text{ mg/L})V_1 = (10.0 \text{ mg/L})(100.0 \text{ mL})$$

$$V_1 = 20.0 \text{ mL}$$

Therefore, the standard is prepared by dispensing 20.0 mL of the 50-mg/L nitrate standard into a 100.0-mL volumetric flask and filling it to the mark with deionized water.

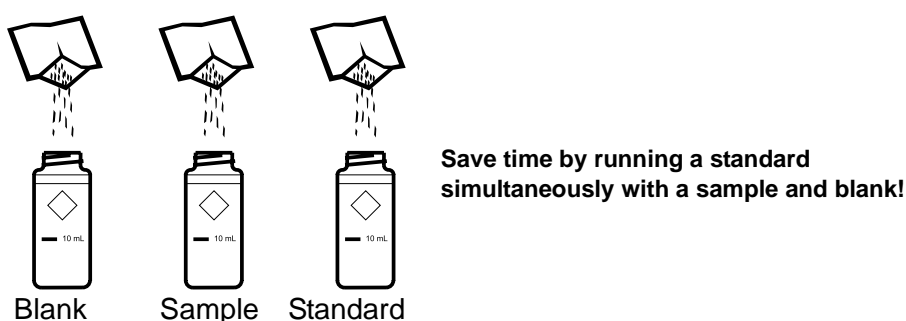
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1.4 Testing Standards

Standards are tested exactly the same way and in the same amount of time as a typical sample. A standard solution can be run right alongside a sample (Figure 3).

When running a test, simply prepare 3 sample cells instead of the usual 1 or 2. Pour the blank into one cell, the sample into the second, and the standard into the third. Add a powder pillow to the necessary cells (refer to the appropriate procedure to determine if a reagent blank is required for a specific parameter), and let the samples react. At the end of the reaction period, zero on the blank and read the sample and standard. Standard results are simultaneously available with sample results.

Figure 3 Standard Solution Alongside a Sample



1.4.1 Frequency of Standard Testing

For labs that are just beginning to use standard solutions for verification, start out by testing a standard solution once per week per parameter tested. If that is successful, increase the frequency of standard testing. Some labs may run a standard in place of a sample 5% or more of the time. It is important to determine a practical standards schedule that meets the needs of the laboratory or plant doing the testing.

Note: It is also necessary to comply with any state or local regulations concerning standard solution testing.

1.4.2 Dealing with Errors

When a 1.00-mg/L standard is prepared and measured, should it read exactly 1.00 mg/L every time it is tested? The reality of chemical measurement is that error happens. Variation in analytical results occurs no matter how carefully the test is performed. There is always variability in the prepared chemistry, the instrument, the test, and the standard itself.

The acceptable amount of variation in a test will differ for different tests and for different labs or plants. For example $\pm 10\%$ may be an acceptable amount of variation for fluoride standards, while $\pm 5\%$ may be an acceptable amount of variation for iron standards. Knowing that variation occurs and knowing how much variation is acceptable will help users keep better track of standard performance. Keeping track of standard performance can help keep a process in control by alerting the analyst to small testing problems before they become larger ones.

2.1 Standard Additions

While most Hach chemistries are compatible with a variety of sample types, there are a few analytical methods that only accurately work with a specific type of sample. Most Hach procedures list many types of samples compatible with the test. It is possible to determine whether a method is compatible with a specific sample by using standard additions. A standard addition (or standard spike) is a method of verifying the compatibility of an analysis method with a particular sample and determining if there are any substances in the sample that will interfere with the analysis method.

To perform a standard spike, first analyze an unspiked sample and record the sample concentration. Next, add a known amount of standard to a known volume of fresh sample. Repeat the test, and check to see if the additional amount of standard was completely recovered.

Total recovery of the spiked standard supports that the sample is compatible with the test. Less than 90% or greater than 110% of recovered spiked concentration may indicate that a substance in the sample interfered with the sample analysis method. The sample may need to be pretreated before it can be analyzed, or an alternate testing method may need to be used.

Step-by-step instructions for the standard additions process are included as part of most Hach procedures under a section titled Accuracy Check, Standard Additions Method.

Example: Iron Standard Additions

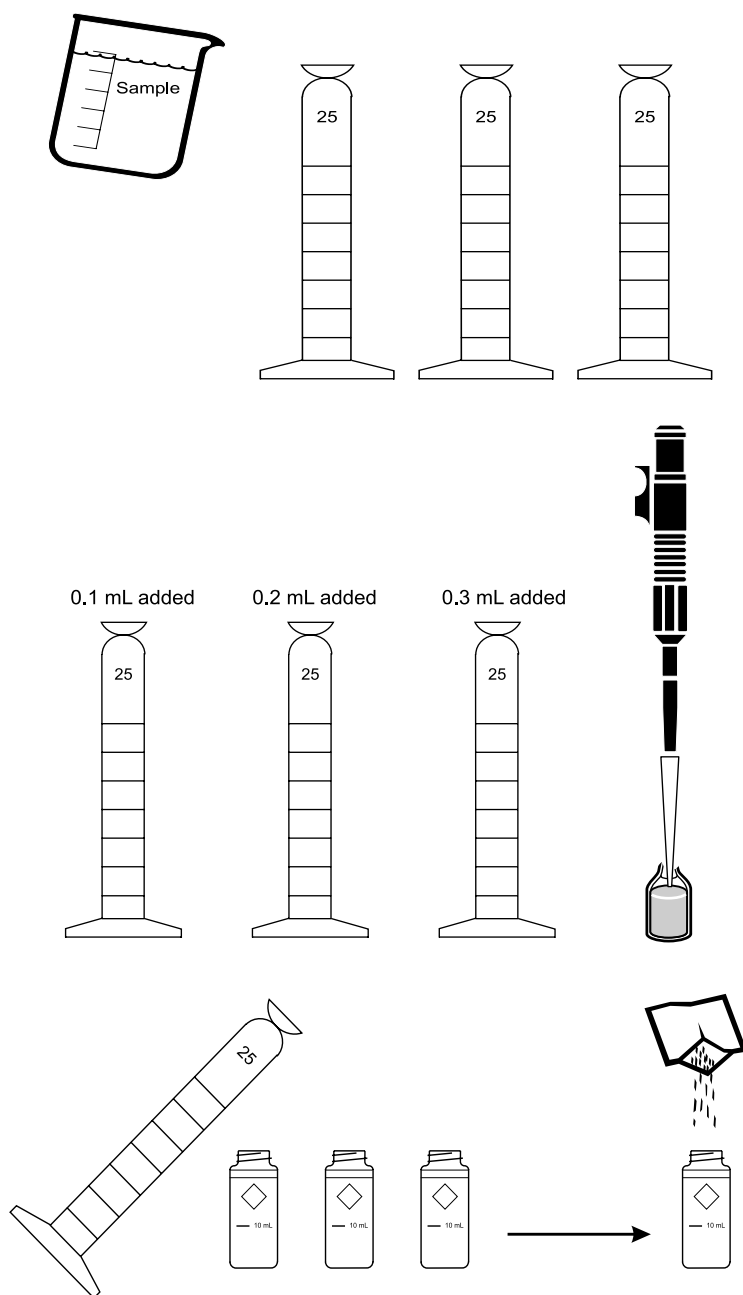
This method follows the standard addition instructions provided in the Iron, FerroVer procedure.

1. Analyze a sample and record its concentration. In this example, the sample has a concentration of 1.00 mg/L iron.
2. Fill three 25.0-mL graduated mixing cylinders with 25.0 mL of sample.
3. Snap the neck off a 50.0-mg/L Iron Voluette® Ampule Standard Solution. Use the TenSette® Pipet to add 0.1, 0.2, and 0.3 mL of standard respectively to the three mixing cylinders.
4. Stopper each cylinder and invert to mix thoroughly.
5. Analyze each spiked sample. Transfer the correct amount of solution to three sample cells and analyze as the procedure indicates. The measured iron concentration should increase by approximately 0.2 mg/L for each 0.1 mL of standard added. If the original sample had a concentration of 1.00 mg/L iron, then the spiked samples should have concentrations of 1.20, 1.40, and 1.60 mg/L iron.

In this example, the three standard spikes are found to have 10% of the correct iron concentrations of 1.20, 1.39, and 1.58 mg/L, respectively. This means that nothing in this sample significantly interfered with the iron test. However, if incorrect concentrations occurred, it may indicate that a sample interfered with the test and that troubleshooting is needed.

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Figure 4 Iron Standards Additions Method



2.2 Calculating Standard Additions

If a Hach standard additions procedure is followed, the expected standard additions concentrations will be stated as part of the instructions. Some Hach spectrophotometers will perform the calculations as part of a Hach program. If an analyst is not following a Hach standards additions procedure, the following formula can be used to calculate the concentration of a sample after it is spiked with standard:

$$\text{Theoretical Concentration} = \frac{(C_u V_u) + (C_s V_s)}{V_u + V_s}$$

Where;

C_u = Original measured concentration of unspiked sample

V_u = Volume of sample to which the spike is added

C_s = Concentration of the standard used to spike

V_s = Volume of standard used to spike

If this equation is used to calculate the standard addition concentrations from the previous iron example:

$$\text{Theoretical Concentration} = \frac{(1.00 \text{ mg/L} \times 25.0 \text{ mL}) + (50.0 \text{ mg/L} \times 0.10 \text{ mL})}{(25.0 \text{ mL} + 0.10 \text{ mL})}$$

When the equation is solved, the theoretical concentration of the 1.00 mg/L sample spiked with 0.1 mL of 50.0-mg/L standard should be 1.20 mg/L (an increase of 0.2 mg/L with each 0.1 mL spike, as indicated in the procedure).

Chlorine (A Special Case)

Chlorine is one of the most common tests that drinking water and wastewater plants perform on-site, since the test must be run immediately after sample collection. The chlorine test is often used to verify the calibration of online analyzers, such as the CL17. It is important to ensure that the test is reading accurately, since the chlorine test is relied on so frequently.

Although the use of standard solutions is highly encouraged for verification of many parameters, it is not the simplest way to verify the accuracy of chlorine measurements. A chlorine standard solution is not the best verification approach because it is difficult to prepare an accurate chlorine standard solution (and a standard is not useful if its exact concentration is unknown). To prepare an accurate chlorine standard solution, all glassware must be chlorine-demand-free and the standard must be diluted with organic free deionized water.

To avoid these problems, the accuracy of the chlorine test can be proven using a modified form of standard additions. Figure 5 illustrates the process of running a chlorine modified standard additions.

To perform chlorine modified standard additions:

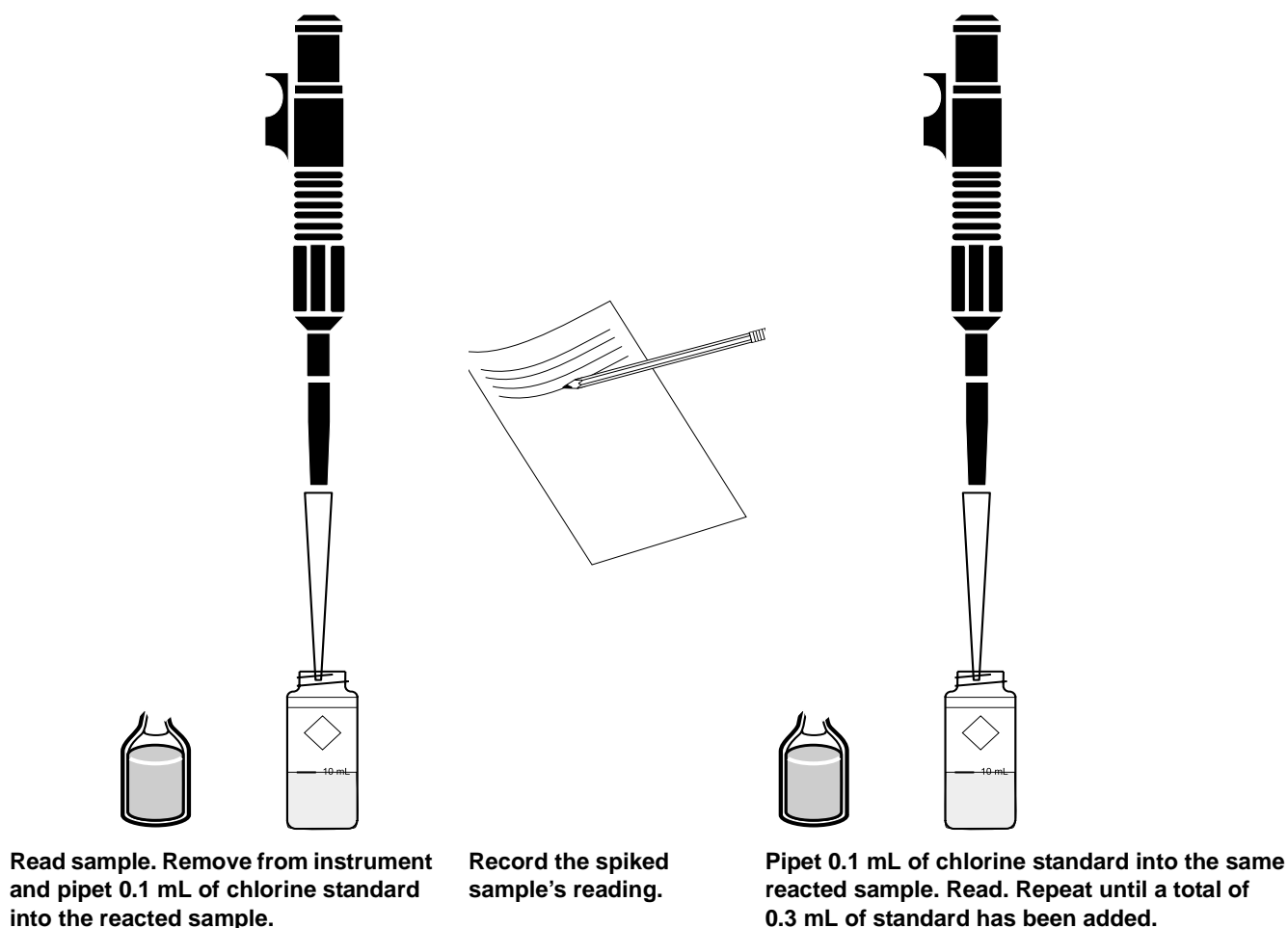
1. Pipet exactly 10.0 mL of sample into a 10-mL sample cell.
2. Run the DPD chlorine test on this sample and record the results.

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3. Open an ampule of chlorine standard solution, 25–30 mg/L and pipet 0.1 mL of standard directly into the reacted sample.
4. Mix thoroughly and read the spiked sample.
5. Pipet another 0.1 mL portion of standard directly into the reacted sample.
6. Mix and read.
7. Repeat with a final 0.1 mL spike.

Record the results. Use the calculations on page 13 to determine what the increase in chlorine concentration should be for each spike. The final volume of sample in the sample cell is 10 mL plus the volume of standard used for the spike. So if 0.1 mL of standard was spiked into the sample, the final volume used in the calculation is 10.1 mL. Note that the 25–30 mg/L chlorine standard solution used in this method actually has a specific chlorine concentration. The exact concentration of the standard is printed on the packaging and should be used in all calculations.

Figure 5 Chlorine Modified Standard Additions Process



Note: A chlorine standard solution can be prepared in the same way. Instead of using the above sample, use 10.0 mL of deionized water. Add a chlorine reagent powder pillow and zero the instrument. Then spike with 0.41 mL chlorine standard and read. Calculate the theoretical standard calculation (see page 13).

This method of standard additions and preparation is only valid for chlorine samples. It is not compatible with other parameters. For other parameters, refer to their appropriate procedure for standard addition steps.

2.3 Quality Control Standards

Another type of standard that an analyst may want to consider testing is a quality control standard (QCS). A QCS is a type of standard that allows users to verify their total analytical system. A QCS can be treated in exactly the same way as a typical sample. Hach offers a variety of quality control standard solutions for the common chemical parameters in drinking water and wastewater. The QCS offered by Hach include standards for Drinking Water Inorganics, Wastewater Influent Inorganics, Wastewater Effluent Inorganics, Hardness (high and low range), Drinking Water Metals (high and low range), and Oxygen Demand.

Quality control standards are useful because they enable the user to verify the entire analytical system and eliminate bias from analytical results. Because the standards can be pH adjusted, digested, or distilled as a typical sample may be, quality control standards are able to verify performance of the entire system—not just the test itself. Correct results can be used to verify the technique of laboratory personnel. Testing a QCS from Hach can also be good practice for analysis of unknown samples that may come from a regulatory agency.

Quality control standards are different from single parameter standard solutions in that the solution contains a mixture of analytes. The nominal concentration of each analyte is printed on the bottle label. Exact concentrations are given on the Certificate of Analysis, which is included with each sample. Certificates of Analysis are available at the Hach website (www.hach.com).

When testing a quality control standard, the standard can be prepared as a typical sample (pH adjusted, digested, or distilled if necessary) and then tested. Standards are formulated so one component in the standard will not interfere with the Hach procedure for another component.

Once the standard has been tested, the analyst can compare their measurements with the values stated on the Certificate of Analysis.

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2.4 Calibration Standards

Standards are often used to verify an instrument's calibration. Standards for pH are known as buffers. Buffers are solutions with known pH values because they contain components that help the solutions resist changes in pH. (In the case of pH, buffers are used for calibration. Typically, calibration is performed with 2 or 3 pH buffers.). Ion selective electrodes (ISE), another type of electrochemical measurement, also requires regular calibration. ISEs are calibrated using standard solutions of the ion being measured.

Formazin or StablCal® (a stabilized form of formazin) standards are used to calibrate both laboratory and online turbidimeters. Prepared StablCal standards can be purchased at the NTU values required for calibration of any Hach turbidimeter. StablCal turbidity standards are also useful for verification of turbidity measurement. StablCal standards are available in a variety of concentrations—a few are even less than 1 NTU.

Turbidimeters and pH meters require regular calibration. Standards must be used to calibrate these instruments. Hach colorimeters and spectrophotometers differ from this because they do not require calibration. The instruments are calibrated at the factory to directly read in concentration units for each analyte. There is no need to perform a calibration that relates absorbance or percent transmittance (%T) to a concentration. In many cases, the calibration in a Hach colorimeter and spectrophotometer will hold for the life of the instrument.

However, there are some cases where a spectrophotometer may need to be calibrated. A calibration may need to be performed if using a non-Hach spectrophotometer that reads in units of absorbance or %T. Many Hach instruments also store User Calibrations and/or custom calibrations that are created by the user. This is necessary if users are running a non-Hach chemistry on a Hach instrument. Finally, users may enter a custom calibration if correcting for wavelength discrepancies or reagent blanks.

2.4.1 Creating a Calibration Curve for a Colorimetric Test

A calibration is the process of establishing a relationship between two properties. In the case of colorimetric measurement, a calibration establishes a relationship between a sample's concentration and the amount of light it absorbs, or absorbance, which is a measurement of color intensity.

Generally, a linear relationship exists between the concentration of a substance in a sample and the amount of light it absorbs. This means, if concentration were plotted versus absorbance, a line would connect the points. This linear relationship in a colorimetric measurement is known as Beer's Law. Beer's Law states:

$$A = abC$$

Where:

A = Measured absorbance

a = Molar extinction coefficient (a constant)

b = Pathlength of sample cell

C = Concentration

Since a, the molar extinction coefficient, is a constant, and b, the pathlength, is a constant, the measured absorbance is equal to a constant multiplied by the concentration of the standard. A calibration curve serves to calculate the value of this constant.

A linear calibration would have the mathematical relationship: $y = mx + b$, where y is the concentration, m is the slope of the line, x is the measured absorbance, and b is the intercept. However, calibration curves for colorimetric chemistries may be more complex. Sometimes, the relationship between absorbance and concentration may be nonlinear (a quadratic or cubic relationship may exist).

2.4.2 Creating a Calibration Curve

To create a calibration curve for a chemistry, prepare at least 5 standard solutions evenly spread across the measurement range of the test. For example, if creating a calibration curve for an iron test that measured from 0-3mg/L, a set of standards may include: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5mg/L standards. A 3.5mg/L standard can be included in the calibration curve in order to assess the effect of an over-range condition on the chemistry. Measurements on the over range point are not needed to calculate the equation of the calibration line. This is especially true if results on the over range standard are inconsistent with the remainder of the calibration curve.

After the standard solutions are prepared, they are ready to be reacted with the chemistry and read on the spectrophotometer (samples in which the analyte of interest adds color to the sample, for example, copper sulfate or industrial dyes may not need any chemistry added to them for calibration and measurement). Zero the spectrophotometer at the chosen wavelength with deionized water. If the chemistry in use has a significant reagent blank, it may be necessary to zero the instrument on a reagent blank rather than deionized water, to create a reagent blank corrected calibration curve. Read the absorbance of each reacted sample at the same wavelength. Record the results for each sample.

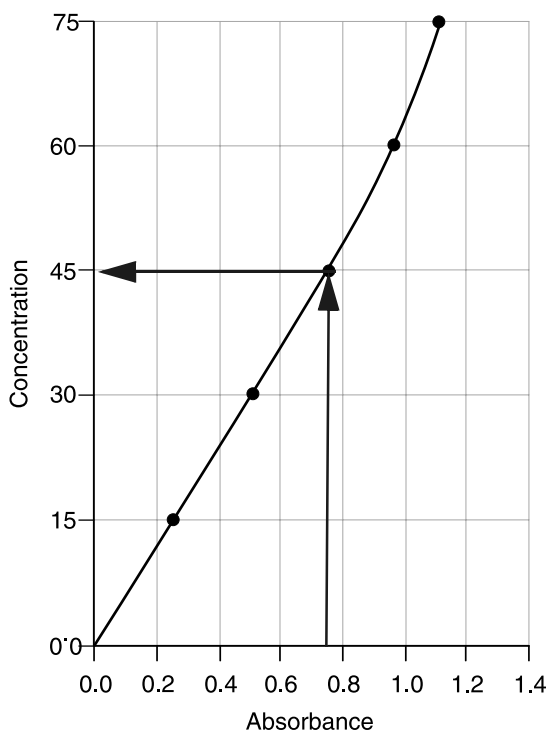
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Once results are recorded, prepare a graph of concentration versus absorbance (concentration on the y-axis and absorbance on the x-axis) and determine the equation of the calibration line. Use this equation to determine the concentration of an unknown when its absorbance is measured. Figure 6 is an example of a calibration curve.

After preparing a calibration curve, it is always a good idea to verify the accuracy of the calibration using a standard solution from a different source from the one used to prepare the original calibration standards.

If using a Hach instrument, it is not necessary to prepare a graph. Most Hach instruments have a User Calibration mode in which the instrument will measure absorbance of a set of standards and determine the relationship between absorbance and concentration. The calibration curve is stored as a User Program. When the calibration is needed, the user can call up the stored User Program and measure samples.

Figure 6 **Calibration Curve**



2.4.3 Wavelength

All colorimetric tests are performed at a specific wavelength of light, which is an important factor in any colorimetric analysis.

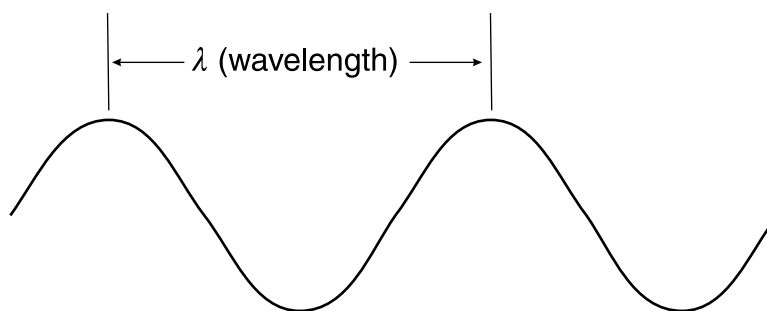
Light is a form of energy that exists as waves (see Figure 7 for a diagram of a wave). The wavelength is the distance between any two peaks or troughs on the wave. Wavelengths can range from the distance of feet (low energy radio waves) to angstroms (gamma rays). Visible light has a wavelength in the range of nanometers (one nanometer is 10^{-9} meters).

Visible light contains the colors of the rainbow: red, orange, yellow, green, blue, indigo, and violet. Red light has a longer wavelength than blue light. Light that is red has a wavelength in the 900nm range. Light that is blue has a wavelength in the 400nm range.

Visible spectroscopy (employed in the measurement of colored samples) operates in wavelengths in the visible light range. Other types of measurements may operate in different ranges of light. Some other types of spectroscopic measurements include infrared, ultraviolet, and nuclear magnetic resonance (uses radio waves).

A monochromator is a part of spectrophotometers and colorimeters. A monochromator selects one specific wavelength of light (or a narrow range of wavelengths) to pass through the colored sample. In the past, a monochromator was as simple as a piece of colored glass. In order to achieve a green colored light, Hach's earliest colorimeters would pass white light through a green piece of glass. Today, a prism, diffraction grating, or LED can function as a monochromator.

Figure 7 Wavelength



2.4.3.1 Selecting the Wavelength

Before the monochromator can be used to select one wavelength for a test, the optimal wavelength for the test must be known. Hach chemists select the optimal wavelength for the test when creating a calibration curve for a Hach chemistry. The wavelength Hach selects is designed to maximize accuracy and sensitivity while reducing the effects of potential interferences. Hach chemistries should be run at the Hach-chosen wavelength for most accurate results. This wavelength can be found in the Hach Water Analysis Handbook or in an instrument procedures manual.

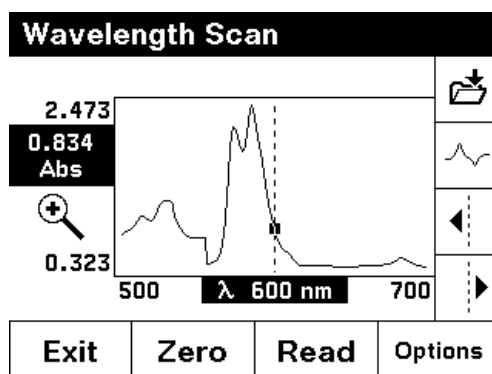
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When creating a calibration curve for chemistries not created by Hach, the user may need to determine the optimal wavelength for measurement. Typically, the optimal wavelength for a colorimetric test is the wavelength at which the sample absorbs the most light. Typically, the optimal wavelength for this is the complementary color to the color of the sample. When the colors are arranged in a color wheel, a color's complement is situated directly across from it. Primary colors are red, yellow, and blue, and complementary colors are mixtures of two primary colors that include orange, green, and violet. Based on this description, the complementary color to red is green. Orange is the complementary color to blue, and indigo is the complementary color for yellow. Chemically speaking, this means that a pink sample (as in the chlorine test) absorbs green light best, its complementary color. A blue sample (as in the phosphate test) absorbs the most yellow-orange light, its complementary color.

Knowing that a sample absorbs the most light of its complementary color, gives some direction in choosing a wavelength for the test. Measure the absorbance of a sample at a variety of wavelengths near its complementary color. In most cases, the test can be run at the wavelength that has the highest measured absorbance.

Another tool that can help with this decision is scanning. A few Hach spectrophotometers are able to scan samples. Scanning is the automatic measurement of absorbance at a range of wavelengths. At the touch of a key, absorbance can be measured at all wavelengths from 400-900nm. The completed scan can be viewed and the wavelength of highest absorbance selected. Figure 8 is an example of a wavelength scan.

Figure 8 **Wavelength Scan**



3.1 Lab Management and Quality Control

Data management is the job of both the analyst and the user. The analyst is defined as the person or group providing analytical results, and the user is the person or group, managing, or interpreting the results. It is important that the user defines the information they are requesting and that the analyst provides the correct information to the users.

The keys to quality data management include documentation and training. Proper documentation and training includes lab management such as record keeping, cleanliness, labware, maintenance, use of standards, stability of reagents, and procedures (choice and training).

3.1.1 Record Keeping

An important aspect of laboratory management is sample tracking. A lab should develop a sample tracking system that tracks samples before, during, and after analysis. The record keeping system should be easily understood and utilized by everyone involved. A good record keeping procedure should be designed to be efficiently processed through the laboratory system while minimizing the actual time spent recording data.

Useful data recorded includes information about the sample and sampling conditions as well as information about the laboratory analysis. Sample and sampling information includes the date the sample was obtained, the names of people who collected the sample, the location where the sample was obtained, sampling comments (any preservation used or weather conditions at the time of sampling), and any internal code assignments for the sample.

Useful laboratory information to collect includes the date of sample analysis, the names of laboratory technicians performing the analysis, analytical results, and analytical comments pertinent to the test (these may include dilutions performed or interferences). Comments should include information that will help the analyst and users understand the analysis that was performed, in the case that there are any discrepancies in results.

Information pertaining to standard solutions used should also be recorded. It is always important to note the date on which a new bottle of standard has been opened. This information can be written directly on the bottle of standard. Results obtained by analyzing a standard should also be recorded, so that standard performance can be tracked over time. Recording standard results can be as simple as writing them in a dedicated notebook. A discussion of other methods to track standard performance is included in the next section.

3.1.2 Cleanliness

Proper cleaning techniques are critical to obtaining maximum accuracy in both sample and standard results. It is suggested that laboratories have a documented procedure for labware cleaning and all persons cleaning labware should be properly trained. Once labware is properly cleaned, it should be stored in a location that prevents contamination from airborne dust or other particles.

Lab Management and Quality Control

Cleaning technique varies depending on the analyte of interest—the optimal cleaning method for analyzing trace organics may not be the same method that is best for metals analysis. Whatever the procedure for cleaning labware is, it is important that the procedure is thoroughly documented and that personnel are properly trained. The following are basic guidelines for routine cleaning, and they may not be relevant for every type of analysis:

- Begin the cleaning process by rinsing glassware with hot tap water.
- Clean the glassware in a solution of water and a laboratory detergent (preferably a phosphate-free detergent).
- Rinse the glassware with an acid solution (either 1:1 Hydrochloric Acid or 6N Nitric Acid), and finally, triple-rinse the glassware with deionized water. Prior to analysis, it is usually best to rinse the glassware with the sample being tested to prevent any cross-contamination or dilution effects.

3.1.3 Labware

The quality of analytical results for both samples and standards depends, in part, on the quality of the labware used in the analysis. For best results, use glassware of the highest quality that is best suited to the application in which it is to be used.

Use clean, Class A glassware to minimize error when preparing dilutions of standards or samples because of its tight volume tolerances. The optimal pieces of glassware to use when preparing dilutions include volumetric flasks and volumetric pipets.

A viable alternative to Class A glass pipets is an accurate volumetric dispenser such as the Hach Tensette pipet. Graduated cylinders are not equivalent alternatives to Class A glassware, although they may be used for the preparation of dilutions if no alternative is available. Be aware that dilutions prepared in graduated cylinders will not have the same degree of accuracy as dilutions prepared using Class A volumetric glassware.

3.1.4 Maintenance

Instruments must be properly maintained to ensure that they continue to meet the performance specifications assigned by the manufacturer. Many Hach instruments include pre-programmed diagnostic procedures that can be used to check instrument conditions. Some automatic checks include stray light checks, wavelength checks, drift checks, and noise checks. Refer to the appropriate instrument manual for more information on automatic instrument diagnostic procedures.

Performance of the instrument can be verified by testing standard solutions. Correct results obtained when testing a standard solution indicates that the analytical system (instrument, reagents, and analyst) is working together properly to produce correct results. Running multiple standards that are read at different wavelengths helps to verify the performance of the instrument throughout its wavelength range.

Determining the precision obtained by running multiple replicates of a standard solution may also aid in assessing instrument performance.

Use caution when assessing instrument performance with standard solutions. When standard solutions are reading correctly, chances are the instrument is performing properly. However, when standards are not reading correctly, this does not necessarily mean that the instrument is malfunctioning. The reading may be due to other factors within the analytical system. If standard solutions do not read properly, further troubleshooting is required. Section 4 on page 35 describes some troubleshooting areas to investigate when standard results are not correct.

The best kind of maintenance is preventative maintenance. For optimal instrument performance, follow any maintenance program and guidelines suggested by the instrument manufacturer. In the majority of cases, following proper preventative maintenance guidelines will help minimize future instrument performance problems.

3.1.5 Use of Standards

Standards are a part of every Hach and EPA method, and are an important part of any laboratory's standard operating procedures.

Standards are used in many different ways, which are described throughout this book. Standards are used to calibrate and to check the calibration of instruments. Standard solutions are run to check and track the performance of the analytical system. Standard solutions are also often used in the training of personnel. Standard additions are used to measure the recovery of a standard that has been spiked into the sample matrix. Standard additions are a useful tool in assessing the presence of interferences in the sample matrix. They can also be useful in assessing the analytical system. Quality control standards can be used to verify interferences and the correctness of standard pretreatment techniques.

Proper standard usage techniques are described in Section 1 and Section 2.

3.1.6 Stability of Reagents

Although Hach's unit-dose reagent packaging is conducive to superior reagent stability, all reagents have a finite shelf life. Many Hach reagents have their expiration dates printed directly on the packaging. Reagents that do not have an expiration date printed on the packaging will have a lot code. The lot code indicates the date on which the reagent was manufactured. The expiration date for the chemical can be established by utilizing this information and the reagent shelf life (available from www.hach.com or by calling Hach Customer Service).

Hach utilizes a Julian lot code system. Hach began using the Julian lot code system in the early 1990s. Previous to this, lot codes consisted of two letters and two numbers. Reagents that have a lot code consisting of two letters and two numbers are unusable, since Hach reagent shelf lives are no longer than 5 years.

A lot code consists of a letter followed by four digits. The letter has no meaning in terms of the manufacture or expiration date. The first digit is the last digit of the year in which the reagent was manufactured. The last three digits (001–365) represent the day of the year in which the reagent was manufactured. For example, a product that has a lot number of A0221 was manufactured on day 221 of the year 2000, or in August of 2000. If the product had a one-year shelf life, it would expire in August of 2001.

Reagent shelf lives are dependent on many factors. One factor is proper storage. Reagents should be stored in a cool, dry location to maximize shelf life. Refrigerate reagents only if indicated on the label. Reagents that are stored at elevated temperatures or in humid locations may experience accelerated degradation and reduced shelf life. Also, if reagents or standards are contaminated, they may be unusable even if they have not expired. Good laboratory practices, such as not introducing a pipet into a bottle of reagent or standard, should be followed to minimize the occurrence of contamination.

Testing a standard solution can assess reagent stability and function. Correct results on a standard indicate that the analytical system, including reagents, is functioning properly.

3.1.7 Procedures

In any standard or sample analysis, it is imperative that the correct analytical procedure is selected and followed. The analytical procedure should account for the concentration range and necessary precision, required regulatory approvals, and the correct chemical form of the analyte. All procedures chosen for analysis should be based on sound chemical principles. If a Hach chemistry is chosen, this is not a concern, since all Hach chemistries are based on sound chemical principles and backed by thorough research and development. Hach procedures should be exactly followed and should not be modified.

Procedures should not compromise the safety of analysts involved. Safe procedures should be chosen over more hazardous procedures, if possible. If a hazardous procedure must be used, proper safety equipment (such as fume hoods, gloves, and goggles) should be provided to the analyst.

Once a procedure is chosen, it should be followed exactly by all personnel involved with chemical analyses. Before a new procedure is included for use on samples, it should be practiced using standards with known concentrations to verify the analytical system. Practicing a new procedure with standards assures the skills of the analysts running the procedure.

3.2 Method Performance

Analysis of samples and standards is required for many different purposes. The main objective of analysis is to obtain one or more analytical results that can be compared to other results or a specific numerical value. Often, the comparison between analytical results, along with other relevant factors, forms a knowledge base that can be used in decision-making.

Accuracy of analytical results, whether on a sample or standard, is a key issue in any quality control program. Decision makers need to make the proper decisions in a timely fashion. Since decisions are based on the analytical data, it is important that any factors that may affect the accuracy of the data are considered.

Analytical results are always subject to errors that can potentially affect the validity of decisions based on those results. Ideally, every analytical result would always be equal to the true concentration in a sample or standard, but this is impossible to achieve.

Analysts can quantify the effects of error when the effects of error are quantified, they can more accurately be taken into account when data is used for the basis of decision-making. Once errors are quantified, steps can be taken to minimize and control the impact on an analysis.

3.2.1 Causes of Error

Uncertainty in chemical measurement arises from systematic and/or random errors. Systematic errors are repeated for every measurement that is made. An example of a systematic error is a reagent blank. Recall that a reagent blank is color added to the sample by the reagent, even when no analyte is present in the sample. A reagent blank can add an error to sample measurements, causing consistently high results. Systematic errors, if identified in the system, can often be controlled and corrected for. For example, the reagent blank value can often be determined and subtracted out from all sample measurements.

Random sources can also cause errors. Random errors are different for each test and may add either positive or negative bias. Random errors may result due to variation in analytical technique and cause random data variation. An example of random error is variation in results due to multiple technicians filling a sample cell to different volumes. Potential sources of random error can be identified, but they can be much more difficult to control than sources of systematic error.

This sounds like error is unavoidable and getting a correct answer is impossible, but this is not completely true. High quality measurements are achievable with attention to detail and technique. Understanding method performance—the method detection limit, sensitivity, precision, and tracking accuracy—will help analysts improve measurement quality.

3.3 Introduction to Statistical Concepts

3.3.1 Sample and Population

The first term requiring definition is population. The user defines the identity of the population. Populations can be either finite or infinite. A finite population is a population that can be clearly defined, such as the number of cars produced in a factory on a given day. An infinite population cannot be clearly defined.

In analysis, it is often impossible or impractical to observe the entire population, especially if it is large. Instead of analyzing the entire group, or population, the analyst examines a small part of the group, known as a sample. For example, instead of surveying the entire population of the United States to determine who is most likely to be elected president, a sample grouping of a few thousand people may be polled. In order for accurate results to be obtained in the poll, the sample must be representative of the entire population. If a sample grouping of 5000 Democrats were polled to determine who was likely to be elected president, the results would probably be biased towards the democratic candidate. If the sample grouping were split more evenly between Democrats and Republicans, reflecting the true nature of the political parties in the United States, the results would likely be more accurate.

In environmental analysis, such as sampling from a lake, the population may be the entire volume of water contained in the lake. However, it is impossible to test all of the water in the lake, so a sample must be obtained. The sample must be collected such that the sample results closely reflect the actual conditions in the lake. Collecting a composite sample, rather than a single grab sample, may help to achieve this.

If the sample is representative of the population, important conclusions can be made from the analysis of the sample. In other words, the sample is an estimate of the population.

3.3.2 Mean, Standard Deviation, and Coefficient of Variation

Once a sample has been analyzed, it is often helpful to determine the mean value. The mean is the sum of all values in a sample divided by the number of values included in the sum:

$$\bar{x} = \frac{\sum_i X_i}{n}$$

Where:

\bar{x} = mean

x = the measured values

n = the total number of values summed

Once the mean has been determined, analysts may find it useful to know how far each data point deviates from the calculated mean. This can be determined by calculating the standard deviation of the sample. Standard deviation is the average variation of values from the mean. If the standard deviation is known, then the outcome of a chemical test can be predicted, using a value referred to as the 95% confidence limit. If 100 tests were run, the results of the test will fall within the 95% confidence limit 95 times.

Standard deviation is a useful value in the determination of confidence limits and in the construction of control charts.

$$S^{n-1} = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

Where:

S^{n-1} = standard deviation

$x - \bar{x}$ = difference between the value and the mean

n = the total number of values measured

Example 1: Calculate the mean and standard deviation of the measurements in Data Set 1.

Data Set 1—11.6, 10.0, 8.4, 9.5, 10.5, 7.7, 10.0, 12.3, 11.4, 8.6

When the mean and standard deviation are calculated for Data Set 1, the mean is found to be 10.0. The standard deviation is found to be 1.50. This means that the average deviation of a data point from the mean is 1.50.

Example 2: Calculate the mean and standard deviation of the measurements in Data Set 2.

Data Set 2—109, 92, 93, 108, 91, 94, 110, 107, 106, 90

When the mean and standard deviation are calculated for Data Set 2, the mean is found to be 100.0. The standard deviation is found to be 8.6.

It may seem that Data Set 1 is more precise because the standard deviation is 1.5, compared to a standard deviation of 8.6 in Data Set 2. However, in order to truly compare the standard deviations of two data sets with two different means, it is necessary to normalize the standard deviation based on the mean of the data set. This can be accomplished by calculating the coefficient of variation, or CV, of the two data sets.

CV is calculated by dividing the standard deviation by the mean and then multiplying the resulting value by 100.

$$CV = (S/\bar{x}) \cdot 100$$

Where:

CV = coefficient of variation

S = standard deviation

\bar{x} = mean

When the CVs are calculated for both data sets, it is found that the CV for Data Set 1 is equal to 15.0% ($1.5/10 \cdot 100$). The CV for Data Set 2 is equal to 8.6% ($8.6/100 \cdot 100$). Although the absolute value of the standard deviation is lower in Data Set 1 than Data Set 2, it is a relatively a larger percentage of the mean, hence Data Set 1 is less precise than Data Set 2.

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3.3.3 Method Precision

Precision is a measure of how repeatable the results of an analysis are. Ideally, if an analysis were run 10 times, the same result would be recorded each time. However, every measurement has some degree of uncertainty. If the same analysis were run 10 times, it is probable that each of the 10 results would be different.

Determining precision for a method is a way of quantifying how repeatable analysis results are for that method. It is an estimate of the average variation in method response and is usually defined as the 95% confidence interval for the stated concentration. For best results, precision should be performed using a standard solution prepared in a deionized water matrix. Precision of sample analysis may differ from that obtained on a standard solution, since the components of the sample matrix are often variable.

There are many different ways that precision can be determined and calculated. For general determination of precision, one can measure 7 replicates of a mid-range standard and then calculate the mean and standard deviation.

For example, to determine the precision of iron method that measures from 0.000 to 3.000 mg/L, one may run 7 replicates of a 1.000 mg/L iron standard. Results on the standard are recorded. In this example, results were found to be: 1.002, 0.997, 1.000, 1.001, 0.998, 0.998, and 1.002 mg/L.

Next the mean and standard deviation are calculated for the data set. The mean is found to be 0.9997 mg/L, and the standard deviation is 0.0021 mg/L. The 95% confidence interval is two times the standard deviation, or 0.0042 mg/L. Using this method, the 95% confidence interval when measuring the 1.000 mg/L standard, ranges from 0.996 to 1.004 mg/L. This means that out of 100 analyses of a 1.000 mg/L standard, 95 percent of the values should fall between 0.996 and 1.004 mg/L. Only 5 out of the 100 analyses should produce results outside this range.

Being aware of a method's precision will help analysts determine the feasibility of using a particular analysis method for sample and standard analysis. Most Hach procedures contain a statement about the precision of a method, as determined by chemists at Hach.

3.3.4 Method Detection Limit

Method Detection Limit (MDL) is another useful tool analysts can use to determine the applicability of analytical methods. Most Hach methods report an Estimated Detection Limit (EDL). However, MDL is analyst-specific and must be determined by the analyst.

In the most general terms, MDL is a measurement of how low a given method can practically measure. The USEPA defines MDL as the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero. The MDL for a method varies from analyst to analyst. Therefore, it is important that each analyst

determines their own MDL based on their unique operating conditions. MDL also does not account for variation in sample composition, so the lowest detectable amount of analyte in a sample may vary from the MDL determined on a standard.

Similar to precision, method detection limit (MDL) can also be defined and calculated in a variety of ways. This book provides one basic method of MDL determination. More information can be found in “Standard Methods for the Examination of Water and Wastewater” or in EPA documentation.

1. Determine the EDL for the method. The EDL is the upper 99% confidence limit for zero concentration based on calibration data used to prepare a calibration curve. Most Hach procedures also contain information about a method's EDL
2. Prepare a standard solution that has a concentration (2 to 3 times) that of the EDL. If the EDL is unknown, it can be estimated based on the range and resolution of a given method.
3. Analyze at least 7 replicates of the standard.
4. Calculate the mean and standard deviation of the results. Determine the MDL by multiplying the standard deviation by the appropriate t value for a 99% confidence interval (this can be obtained from a statistical table).

For example, to determine an MDL for Hach's FerroZine iron method, the first piece of information required is the EDL for the method. By referring to the FerroZine procedure in the manual, the EDL is found to be 0.003 mg/L. Next, a standard (2 to 3 times) the concentration of the EDL is prepared. In this case a 0.010 mg/L standard is prepared (approximately 3 times the EDL). Eight replicates of the standard are analyzed. Results on the standard were found to be: 0.009, 0.010, 0.009, 0.010, 0.008, 0.011, 0.010, and 0.009.

Next the mean and standard deviation are calculated for the data set. The mean is found to be 0.0095 mg/L. The standard deviation is determined to be 0.0009 mg/L. To calculate MDL, the standard deviation is multiplied by Student's t value, which is 2.998 in a situation with 8 replicates. When this is done, the MDL is found to be 0.003 mg/L. In this case the EDL appears to be a fair estimate of the MDL. However, the actual MDL can differ significantly from the EDL and between analysts.

3.4 Control Charts

Principles Behind Control Charts

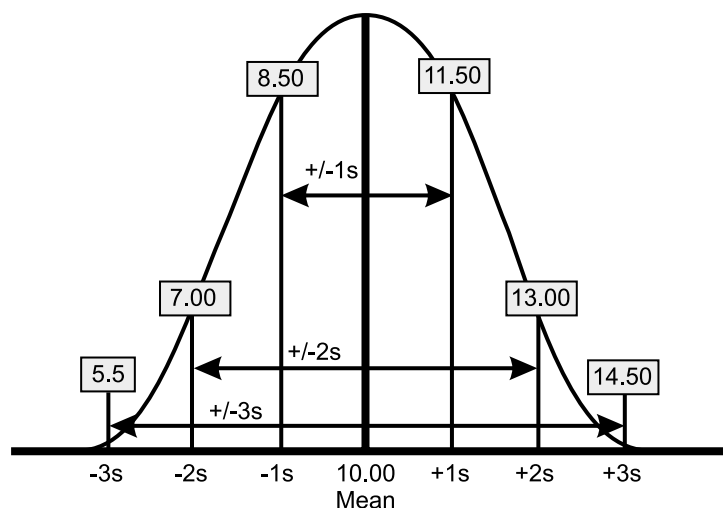
Recall from the previous discussion of statistical principles that a sample is a set of data that represents a certain population (as long as the sample obtained is a true representative sample). If the values of the population were plotted on graph paper, the individual values would be distributed in a bell shaped curve, known as a Gaussian or normal distribution curve. The boundaries of the curve are described by the mean and standard deviation of the sample values.

Figure 9 is a normal distribution for a set of data (11.6, 10.0, 8.4, 9.5, 10.5, 7.7, 10.0, 12.3, 11.4, and 8.6.) The mean of the data set is 10.0, and the standard deviation is 1.50. Two standard deviations are equal to 3.00, and three standard deviations are equal to 4.50.

The mean is located at the peak of the normal distribution curve. As labeled on the curve, one standard deviation ranges from 8.50 to 11.50, or ± 1.50 about the mean. Two standard deviations range from 7.00 to 13.00 or ± 3.00 about the mean. Three standard deviations range from 5.50 to 14.50, or ± 4.50 about the mean.

A fixed percentage of values fall within each standard deviation. Sixty-eight percent of the values lie within one standard deviation of the mean, 95% of the values lie within two standard deviations of the mean, and 99% of the values lie within three standard deviations of the mean. The 95% confidence limits are the values that define the area of two standard deviations about the mean. This principle is used to formulate a control chart.

Figure 9 Normal Distribution



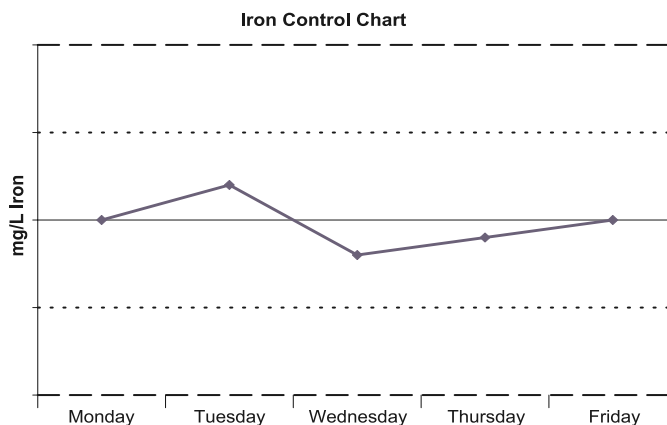
Definition of Control Chart

A control chart is a visual tool used to aid in tracking standard performance. Each time the analyst runs a standard solution, results are plotted on the control chart. A control chart is a graph of standard results plotted over a period of time. Control charts are a quick and easy visual way to track a lab's performance based on running standard solutions. Figure 10 is an example of a typical control chart for a lab's iron standard solution results. The vertical axis of the chart is for test results and the horizontal axis of the chart stands for time. In Figure 10 the vertical axis is labeled mg/L Iron, and the horizontal axis is labeled with the weekdays.

There are also a few horizontal lines drawn across the graph in various locations. These lines have specific meanings. The dotted lines are drawn at ± 2 standard deviations of the standard value. These lines indicate the Upper and Lower Warning Limits. When standard results approach these values, it is an early warning signal that there may be a problem with the test. The dashed lines are drawn at ± 3 standard deviations of the standard value. These lines indicate the Upper and Lower Control Limits. When standard results reach the Control Limits, this usually indicates some sort of problem with the test.

Random variation in standard results is expected. Random variation means that the variation does not have a predictable pattern. From day to day measured values may increase or decrease, but the change in values is not regular. If the variation in values is not random — if it seems to trend up or down over a period of time — this indicates that there may be a problem with the analysis that requires troubleshooting. Control charts help users see when troubleshooting is required—before a crisis occurs.

Figure 10 Normal Control Chart



Types of Control Charts

Figure 10 is a control chart for results obtained by repeatedly analyzing a 1.00-mg/L iron standard. There is some variation around 1.00 mg/L, but there is no clear trend in the variation. This is considered normal variation. This control chart indicates that the user is performing the test correctly, the instrument and reagents are working correctly, and the sample analysis is likely correct.

Figure 11 is another control chart for results of a 1.00-mg/L iron standard. Note the upward trend in the results over time. From Wednesday to Friday, the standard values regularly increase. This indicates that there could be a problem with the analysis. Since results of a standard analysis are high, results of a sample analysis may also be falsely high, leading to unnecessary treatment measures in the plant. This control chart indicates that it is time to troubleshoot the iron test before it causes a larger problem.

Figure 12 is also a control chart for results of a 1.00-mg/L iron standard. In this control chart, the variation is random but a bit more extreme. Iron values exceed the Warning Limits, and there are no clear trends in either direction (increasing or decreasing). This may be an indication of poor technique. If the trend does not improve, troubleshooting is necessary to reduce variability and ensure that results are correct.

Figure 11 Control Chart (Variation)

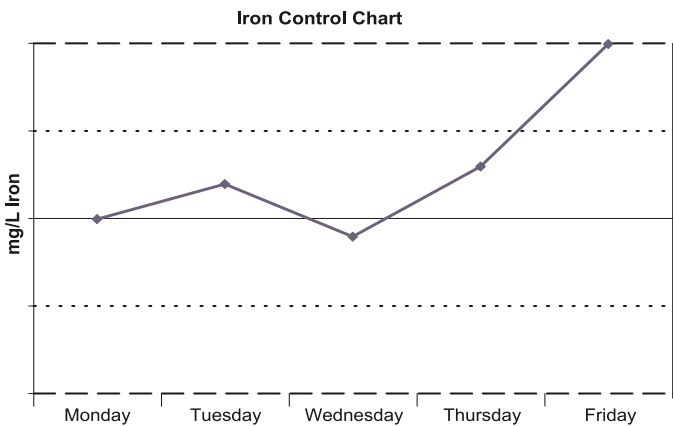
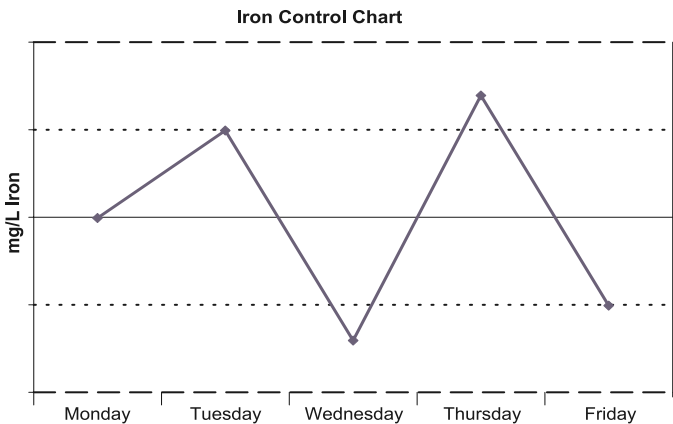


Figure 12 Control Chart (Inconsistent Results)

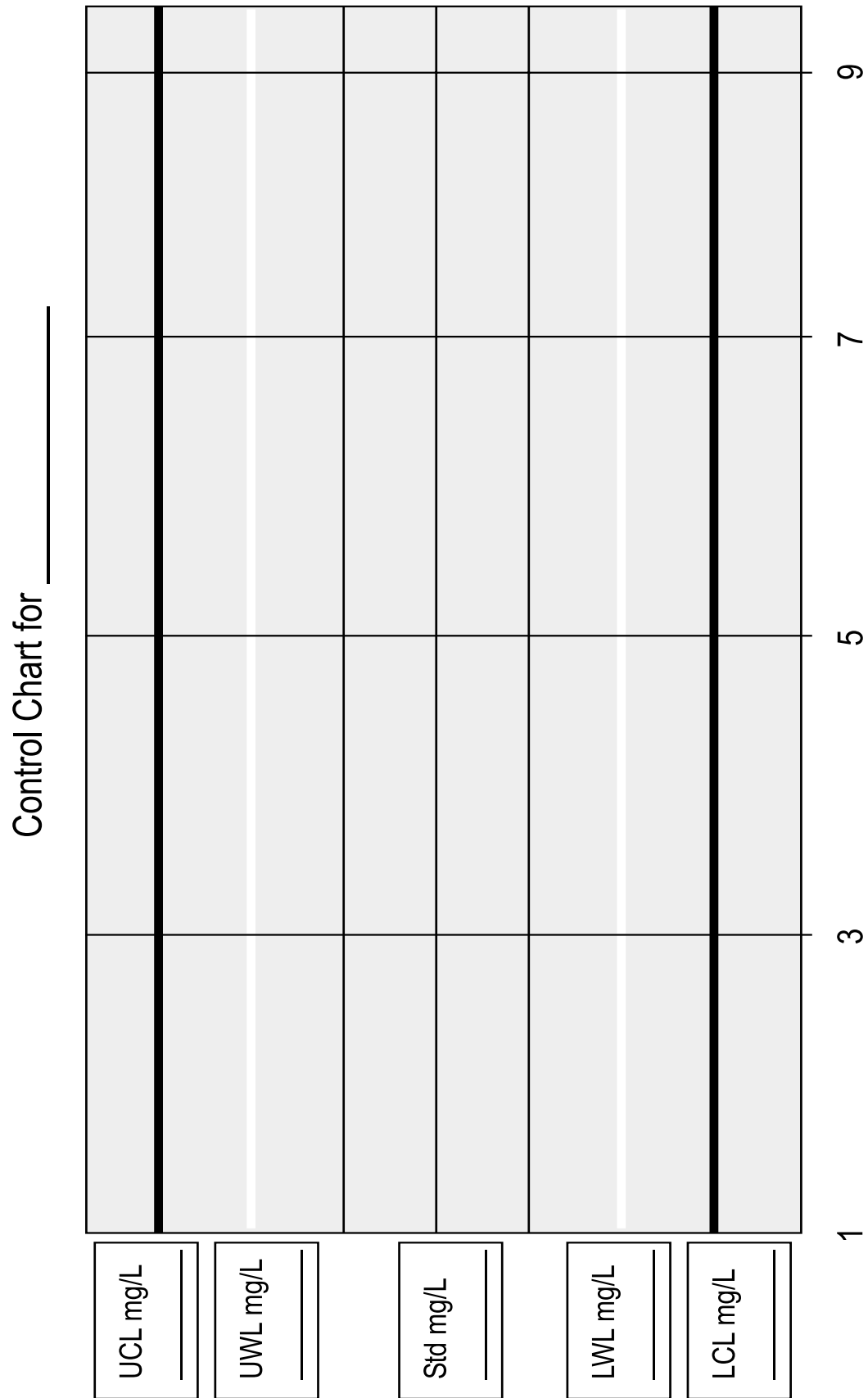


3.4.1 Creating A Control Chart

The blank control chart is intended for use as a master control chart from which the user can make copies for use in the lab. Follow these easy steps to customize the blank control chart (Figure 13):

1. Label the horizontal axis of the chart with the dates the standards are to be tested.
2. Label the vertical axis with the test's units (i.e. mg/L iron). Place the standard concentration in the middle of the vertical axis. Label the axis so the Upper and Lower Warning Limits are 2 standard deviations from the standard concentration and the Upper and Lower Control Limits are 3 standard deviations from the standard concentration. For example, if the iron standard concentration is 1.00 mg/L, and one standard deviation is 0.05, then the Warning Limits are ± 2 standard deviations or 0.90 and 1.10 mg/L, and the Control Limits are ± 3 standard deviations or 0.85 and 1.15 mg/L.
3. Test the standard solution regularly. Weekly is a good frequency to start with. For a control chart to be valid, the measured standard concentration must remain consistent.
4. Plot results on the chart. Draw a line to connect the points.
5. Post the control chart in the lab, where it can be easily viewed. Use the control chart as assurance that the test is working correctly.

Figure 13 Control Chart Template



Ideally, standard solutions would always read correctly and the results of every test run would be accurate. However, this is not always the case. What happens when a standard solution, standard additions, or quality control standard does not read correctly?

4.1 Troubleshooting Standards

The first step in troubleshooting is to take a look at how the standard measurement compares with the actual standard concentration. Typically, the measured standard concentration should read within 5–10% of the actual standard concentration. The acceptable amount of variation can be different depending on the test method and instrument being used. If the standard measurement is within this range, there may be minimal error in the measurement, but it is within the expected range of scatter for the test. If the standard measurement is outside this range (or outside the UCL or LCL on a control chart), it is time to start troubleshooting the cause of the problem. Laboratory or local regulatory agencies may also have specific guidelines concerning the acceptable amount of variation in a standard measurement. It is important for the analyst to follow the recommendations of their laboratory or local regulatory agency.

If there is error in the measurement, it is a good idea to repeat the test. Make sure the error is repeatable and not an isolated incident. Repeating the test once takes little time and is very helpful in troubleshooting.

If a quality control standard or standard additions is being measured, the first step to troubleshooting is to simplify. Run a standard solution and check the results. If the standard solution measures correctly, it is likely that the instrument and reagents are working correctly, and the analyst is running the test properly. If this is the case, the problem is likely something specific to the quality control standard or standard additions procedure. If the standard solution does not measure correctly, stop there and begin troubleshooting. Start troubleshooting the instrument, reagents, and procedure in order to pinpoint the problem.

Use the following questions to aid in troubleshooting a problem with an analytical method:

Instrument Troubleshooting

- Is the correct program being used for the parameter being tested?
Refer to the instrument's procedures manual to check the program number.
- Is the test being run at the proper wavelength?
Results will be incorrect if a test is not run at the proper wavelength.
- Is the answer displayed in the correct chemical form?
For example, phosphate readings can be expressed as mg/L PO_4^{3-} or mg/L P. The same measurement can be described in two different ways, much like miles/hr and kilometers/hr are two different ways of expressing speed. Be sure the chemical form displayed on the instrument matches the form the standard is expressed as.

Troubleshooting

- Was the instrument zeroed on the proper solution?
The instrument is zeroed on standard for some procedures, while others require the instrument to be zeroed on a reagent blank.
- If the reagent blank adjust feature is being used, is the reagent blank value stored for the current procedure? Is the stored reagent blank value correct for the current lot of reagents?
- Is the instrument's standard adjust feature being used?
If so, turn it off and return to the instrument's default calibration to check the standard. Additionally, if an instrument has been calibrated by the user, switch the instrument back to the default calibration or use the Hach program when troubleshooting.
- Is the dilution factor feature in use?
If so, be sure that the multiplication factor is entered correctly, or turn the feature off and correct for any dilution manually.
- Is the instrument regularly maintained? Is there any reason to suspect a problem with the instrument?
Hach instruments do not typically require recalibration. However, if an instrument has been dropped or is routinely handled roughly, it is possible that optical components could fall out of alignment.

Reagents and Standard Solution Troubleshooting

- How old are the reagents? Are the reagents within their expected shelf life?
If reagents have expired, they should not be used for testing standards or samples.
- How have the reagents been stored?
Extreme temperature, humidity, or contamination can affect shelf life. Reagents exposed to extreme conditions will usually have a shorter than expected shelf life. Reagent performance can be verified with a standard solution.
- How old is the standard solution, is it within its expected shelf life?
Standards packaged in plastic bottles have a limited shelf life due to the permeability of plastic. Standards packaged in glass ampules have a slightly longer shelf life. Dilutions prepared from concentrated standard solutions should be prepared immediately prior to use.
- Could the standard be contaminated?
Pipets and other objects should never be placed into a bottle of standard. Always dispense standard into a beaker, and pipet out of the beaker. If questions arise, a new bottle of standard could be used to see if results change.
- Are the proper reagents being used for the sample size?
For example, are using 10-mL reagents being used with a 10-mL sample and 25-mL reagents with a 25-mL sample? Refer to the procedures manual for the correct reagent catalog numbers for a specific test.

Procedure Troubleshooting

- Is the procedure being followed correctly?
Be sure to follow all the steps, including the timers, exactly as written. Many procedures contain important notes, either in front or as part of the steps. Read the entire procedure thoroughly before attempting to run the test.
- If the standard was diluted from a concentrated stock solution, was the dilution performed properly?
It may be beneficial to repeat the dilution and run the test again. Contamination or improper technique can affect the accuracy of the prepared standard.
- Is the glassware used for the test clean?
A dilute acid solution (nitric or hydrochloric acid) may be needed to thoroughly clean glassware (be sure to rinse well with deionized water and sample after washing with acid). Sometimes a reagent rinse will aid in cleaning glassware completely. This is especially helpful in the phosphate test. To run a reagent rinse, fill the cell with deionized water and add 2-3 reagent pillows. The reagents will react with any analyte present in the sample cell and remove it from the system. After performing a reagent rinse, be sure to rinse the cell thoroughly with deionized water, followed by sample, before testing.
- Are the correct sample cells being used for the procedure?
Some instruments can accept many different sample cells. Make sure that the right one is being used for the test. Additionally, be sure that the program selected on the instrument corresponds with the cell in use.

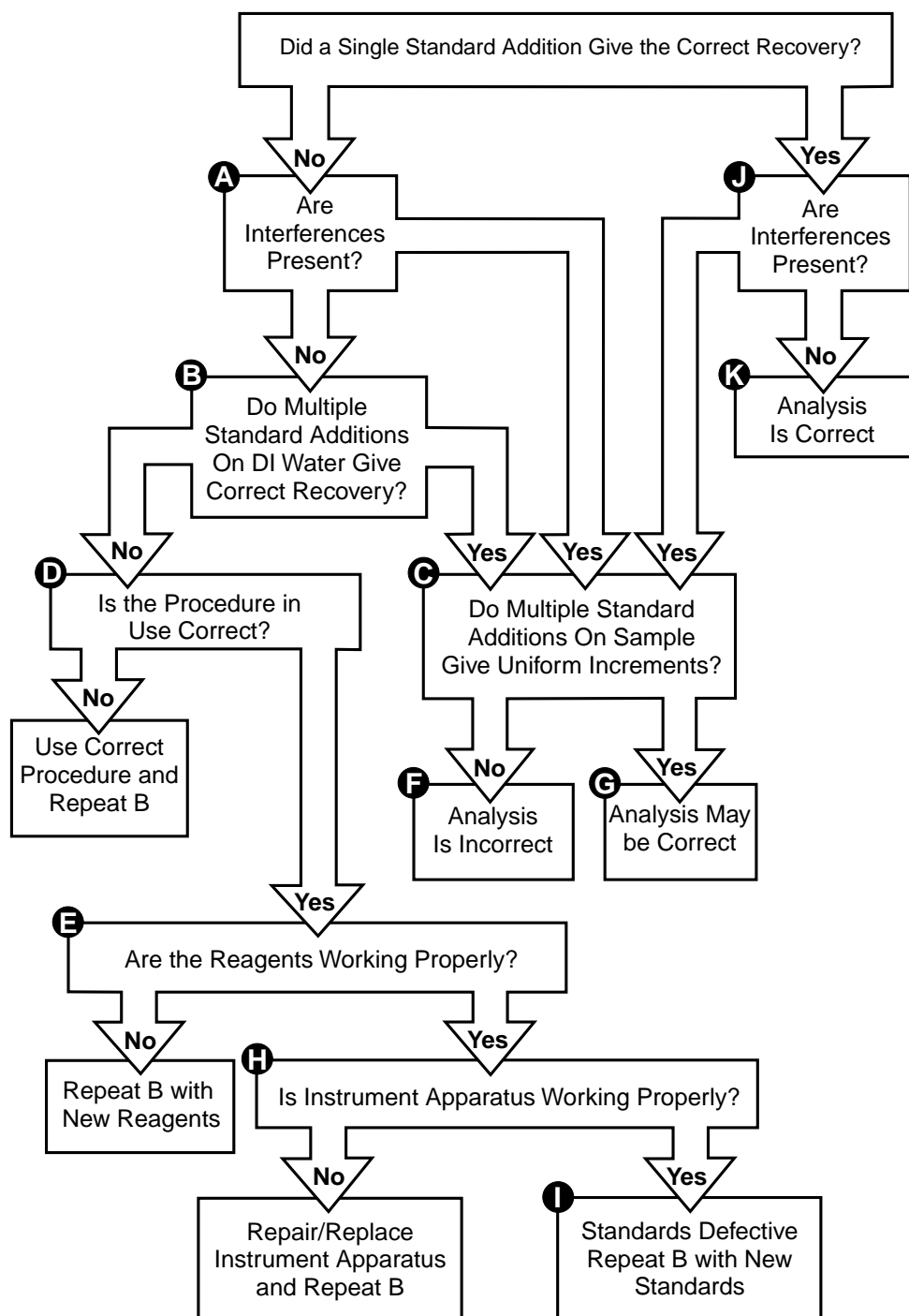
If a standard solution measures correctly but a standard addition does not work with a sample, try spiking the standard into deionized water. If the deionized water spike works correctly but the sample does not, the original sample may contain an interference. The original sample may need to be diluted in order to obtain a correct reading. Dilution serves to decrease the concentration of analyte in the sample as well as decreasing the concentration of potential interferences. If dilution does not improve the interference, a more advanced technique may be needed, or a new method may be selected.

The decision tree displayed in Figure 14 presents a logical flow of questions to use when troubleshooting standard additions.

If a quality control standard does not read correctly, there may be a problem with the sample preparation. Check to see that the sample pH is adjusted correctly. If the quality control standard was digested, distilled, or diluted, be sure that the preparation technique was performed properly.

If problems persist after considering these troubleshooting ideas, and users are still having problems getting the correct reading on a standard, standard additions, or quality control standard, contact Hach for more assistance.

Figure 14 Standard Additions Decision Tree



Additional Information

Additional information about testing samples and standards is available on the Hach website at www.hach.com. Instrument and procedures manuals, technical documents, and Certificates of Analysis and MSDS are also available on the website.

Absorbance—A measure of the amount of light absorbed by a sample.

Analyst—A person who examines a system.

Analyte—The sample constituent whose concentration is sought in a chemical analysis.

Bias—Favoring some outcome over others due to a testing error.

Calibration—The process of establishing a relationship between two properties, for example adsorption and concentration.

Calibration Curve—Graphical representation of a calibration.

Coefficient of Variation—Standard deviation normalized to the mean of a data set $[(SD/mean)*100]$, also known as relative standard deviation.

Diluted—A solution that is less concentrated.

Electrochemical—Analysis method that uses electrical measurement to measure a chemical property (for example pH or conductivity) see also electrochemistry.

Electrochemistry—The science of electricity and chemistry.

Estimated Detection Limit—Defined by the EPA as the upper 99% confidence limit for zero concentration based on calibration data used to prepare a calibration curve, which is the starting point for Method Detection Limit (MDL) determination. Unlike MDL, Estimated Detection Limit is not user specific.

Interferences—Chemical whose presence in a solution affects the accuracy of a chemical analysis.

Mean—The average value of a set of numbers.

Meniscus—The curvature of the surface of a column of liquid confined in a narrow tube.

Method Detection Limit—In general, a measurement of how low a method can practically measure for a given analyst. Defined by the EPA as the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero.

Monochromator—A monochromator produces light of one color. A monochromator may be composed of prisms, diffraction gratings, LEDs, or a piece of colored glass

Over-range—Solution that contains more analyte than a test method is able to measure, for example a 3.5 mg/L iron sample would be considered an over-range if measured using a method that only measures up to 3 mg/L iron.

Pathlength—the length of a path taken by light through a sample.

Glossary

Percent Transmittance—Spectrophotometric measurement of the percent of the incident light passed through a colored solution (a clear solution would transmit 100% of the light passed through it, while a colored solution would transmit less).

Pipet—A device for measuring and transferring small amounts of liquid.

Reagents—A chemical substance, added to a sample to detect or measure substances in the sample.

Reagent Blank—Any color imparted to a sample due to the reagents, not the analyte. For example, Hach's phosphate reagents typically have a slight reagent blank. Even when there is no phosphate in a sample (like Deionized water) some color will exist due to the contribution of color from the reagents.

Spectrophotometer—An instrument for measuring the amount of light absorbed by a sample.

Titration—an analysis method that involves adding a known amount of one substance to measure an unknown amount of another substance that reacts with the first in some complete or known fashion.

Titrimetric—analyzed by titration, see titration.

Turbidimetric—a measurement method used to measure the turbidity (or clarity) of a solution, turbidimetric methods measure the amount of light scattered at a 90 degree angle to the incident light source, also called nephelometric.

Verification—to determine the accuracy of an experiment by comparison or reference.

Wavelength—the distance between two corresponding peaks of a wave of light, heat, or other form of energy.

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