

Contents

1.1	Introduction
1.2	FDA Law and Regulations
1.2.1	FD&C Act and Amendments
1.2.2	Other Acts Enforced by FDA
1.2.3	Code of Federal Regulations
1.2.4	Federal Register
1.3	Analytical Methods
1.3.1	Official Compendia
1.3.2	Specialized Manuals
1.3.3	Other Method Sources
1.3.4	Method Validation Information
1.4	The Sample
1.4.1	FDA Sample Requirements
1.4.2	Sample Accountability
1.4.3	Reporting Sample Analysis
1.4.4	FACTS
1.5	Additional Records
1.5.1	Notebook
1.5.2	Training Progress Report
1.6	Other Information
1.6.1	Administrative and Regulatory Procedures
1.6.2	Laboratory Safety
1.6.3	Quality Assurance
1.7	General References
1.7.1	Handbooks and Indexes
1.7.2	Statistics
1.7.3	Analytical Chemistry
1.7.4	Instrumentation

- 1.8 **Orientation Exercises**
- 1.8.1 Analytical Balances
- 1.8.2 pH Meters
- 1.8.3 Volumetric Glassware
- 1.8.4 Standard Solutions
- 1.8.5 Thermometers (optional)
- 1.8.6 Microscopes (optional)
- 1.9 Appendix - Answer Key**
- 1.10 Document/Change History**

Appendix - Training Progress Report (Form 1A)

Appendix - Training Exercise Review and Completion (Form 1B)

1.1 Introduction

The topics and exercises in this section are fundamental. They are intended for use during initial training of analysts and aides in order to provide a basic understanding of the laboratory's role in consumer protection.

Other sections of this manual cover special training and the Internet courses at Office of Regulatory Affairs University (ORAU) provide training for approximately the first year of employment with FDA. These web-based courses will compliment the laboratory training in identified areas such as the Essentials of an Effective Calibration Program, Hazardous Waste Determination, etc.

The first year is an important one for career employees in the Federal service. During this period employees learn how to serve best in their role of protecting the public; a role for which no educational institution fully prepares them. During this first year new employees are carefully evaluated by their supervisors. The supervisor and laboratory director are responsible for keeping the FDA field scientific staff at the high level of competence and dedication it has historically demonstrated.

Exercises in this section depend on the extensive use of reference material by the trainee. The exercises reference many sources of information. Although most are relatively common, other sources may have to be substituted and supplemental material added. The trainee is encouraged to use the laboratory or district library, the Internet, the FDA Intranet, and other information sources.

1.2 FDA Laws and Regulations

The Federal Food, Drug, and Cosmetic (FD&C) Act, which is the reason for the existence of the Food and Drug Administration, provides legal authority for almost all FDA activities. This Act and some of its amendments as well as a number of other acts are enforced by FDA. The analyst is to be familiar with these and also familiar with two official government publications, the Code of Federal Regulations and the Federal Register.

1.2.1 FD&C Act and Amendments

The FD&C Act, as amended, with its general and enabling regulations, provides the basic authority for the majority of our regulatory operations. The Act is republished periodically in booklet form. The Act will be discussed with the analyst by their training supervisor and, later, in greater detail at FDA training sessions. It is important that the analyst acquire an understanding of its provisions because they will play a significant part in decisions the analyst will make during his or her career.

The Act, which was passed by Congress in 1938, replaced the Food and Drug Act passed in 1906. Since 1938, the Act has been amended a number of times. Among the amendments are the following:

INSULIN (1941) AND ANTIBIOTIC AMENDMENTS (1945 et seq.). Requires FDA to batch-certify insulin and antibiotic drugs before they are placed on the market.

MILLER AMENDMENT (1948). Gives FDA jurisdiction over products that become adulterated or misbranded after interstate shipment.

DURHAM-HUMPHREY AMENDMENT (1951). Makes it unlawful to dispense a drug bearing the Rx legend without a prescription or to refill a prescription without authorization from the prescriber.

FACTORY INSPECTION AMENDMENT (1953). Permits FDA inspection, after written notice, without a warrant and without permission of the owner.

PESTICIDE CHEMICAL AMENDMENT (1954). Requires the manufacturer of a pesticide chemical to obtain a tolerance from EPA for the chemical in or on specified raw agricultural products. The burden for proving that residue levels are safe is placed on the manufacturer. FDA is responsible for enforcing the tolerance established by EPA.

FOOD ADDITIVES AMENDMENT (1958). Requires premarketing clearance of food additives whose safety is not generally recognized. Burden for proving that a food additive is safe for its intended use is placed on the manufacturer.

COLOR ADDITIVES AMENDMENT (1960). Requires that conditions for safe use of a color additive be established by regulation. Burden for proving safety is placed on the manufacturer.

DRUG AMENDMENTS (1962). Requires, among other provisions, that manufacturers prove their new drugs to be effective as well as safe and gives FDA authority to regulate prescription drug advertising.

DRUG LISTING AMENDMENTS (1972). Requires manufacturers and processors of drugs to submit a list of all drugs manufactured or processed for commercial distribution. Among other provisions is the requirement that a copy of the labeling for each drug be submitted.

MEDICAL DEVICE AMENDMENTS (1976). Provides for the safety and effectiveness of medical devices intended for human use. Devices are to be classified and, depending on classification, may require a performance standard or premarket approval.

INFANT FORMULA AMENDMENT (1980). Establishes nutrient requirements for infant formulas and quality control requirements for their manufacturers.

1.2.2 Other Acts Enforced by FDA

TEA IMPORTATION ACT (1897, Repealed 1996). Provides for standards of purity, quality, and fitness for teas and requires the examination of each imported shipment.

FDA FEDERAL IMPORT MILK ACT (1923). Regulates importation of fluid milk products from foreign producers.

FILLED MILK ACT (1927). Prohibits shipment of filled milk in interstate or foreign commerce. Filled milk is defined as any milk, cream, or skimmed milk to which has been added fat or oil other than milk fat to produce a product in imitation of or in semblance of milk, cream or skimmed milk.

FEDERAL CAUSTIC POISON ACT (1927). Specifies warnings and precautionary statements on labeling of certain household caustic preparations. Primarily a labeling act.

FAIR PACKAGING AND LABELING ACT (1966). Prohibits the use of unfair or deceptive methods of packaging or labeling of certain consumer commodities.

RADIATION CONTROL FOR HEALTH AND SAFETY ACT (1968). Protects the public from unwanted exposure to radiation from electronic products, e.g., color TV sets, microwave ovens, and X-ray machines.

PUBLIC HEALTH SERVICE ACT (1944). Areas of this act enforced by FDA are:

- Regulation of interstate sales of biologics, such as vaccines, serums, and blood, to assure purity, safety, and potency.
- Regulation of pasteurized milk and shellfish handling.
- Regulation of food service sanitation and food, water, and sanitary facilities used by interstate travelers on common carriers.

FEDERAL ANTI-TAMPERING ACT (1983). Establishes criminal penalties for involvement in tampering with a consumer product.

ORPHAN DRUG ACT (1983). Encourages development and marketing of drugs for rare diseases by offering incentives to manufacturers.

DRUG PRICE COMPETITION AND PATENT TERM RESTORATION ACT (1984). Establishes criteria for extending patents on drug products, methods of use, and methods of manufacturing.

THE PRESCRIPTION DRUG MARKETING ACT (1988). Bans the diversion of prescription drugs from legitimate commercial channels. Congress finds that the resale of such drugs leads to the distribution of mislabeled, adulterated, subpotent, and counterfeit drugs to the public. The new law requires drug wholesalers to be licensed by the states; restricts reimportation from other countries; and bans sale, trade or purchase of drug samples, and traffic or counterfeiting of redeemable drug coupons.

NUTRITIONAL LABELING AND EDUCATION ACT (1990). Requires all packaged foods to bear nutrition labeling and all health claims for foods to be consistent with terms defined by the Secretary of Health and Human Services. The law preempts state requirements about food standards, nutrition labeling, and health claims and, for the first time, authorizes some health claims for foods. The food ingredient panel, serving sizes, and terms such as "low fat" and "light" are standardized.

SAFE MEDICAL DEVICES ACT (1990). Requires nursing homes, hospitals, and other facilities that use medical devices to report to FDA incidents that suggest that a medical device probably caused or contributed to the death, serious illness, or serious injury of a patient. Manufacturers are required to conduct post-market surveillance on permanently implanted devices whose failure might cause serious harm or death, and to establish methods for tracing and locating patients depending on such devices. The act authorizes FDA to order device product recalls and other actions.

MAMMOGRAPHY QUALITY STANDARDS ACT (1992; in 1998 was reauthorized until 2002). Requires all mammography facilities in the United States to be accredited and federally certified as meeting quality standards effective Oct. 1, 1994. After initial certification, facilities must pass annual inspections by federal or state inspectors.

DIETARY SUPPLEMENT HEALTH AND EDUCATION ACT (1994). Establishes labeling requirements, provides a regulatory framework, and authorizes FDA to promulgate good manufacturing practice regulations for dietary supplements. This act defines "dietary supplements" and "dietary ingredients" and classifies them as food. The act also establishes a commission to recommend how to regulate claims.

FOOD AND DRUG ADMINISTRATION MODERNIZATION ACT (1997). Reauthorizes the Prescription Drug User Fee Act of 1992 and mandates the most wide-ranging reforms in agency practices since 1938. Provisions include measures to accelerate review of devices, regulate advertising of unapproved uses of approved drugs and devices, and regulate health claims for foods.

FOOD AND DRUG ADMINISTRATION ACT (1998). Officially establishes FDA as an agency of the Department of Health and Human Services, with a Commissioner of Food and Drugs appointed by the President with the advice and consent of the Senate, and broadly spells out the responsibilities of the Secretary of DHHS and the Commissioner for research, enforcement, education, and information.

FDA has some enforcement or regulatory authority under a number of other statutes:

- Comprehensive Drug Abuse Prevention and Control Act
- Controlled Substances Act
- Federal Meat Inspection Act
- Poultry Products Inspection Act
- Egg Products Inspection Act

1.2.3 Code of Federal Regulations

The Code of Federal Regulations (CFR) is an official government publication that codifies the general and permanent rules (regulations) issued by the Executive Branch and its agencies to implement the laws they enforce. It is revised annually. FDA regulations are coded under Title 21 as "21 Food and Drugs." References to the CFR are written as "21 CFR" with part, section, and paragraph numbers following, e.g., "21 CFR 121.3(d)." Criminal penalties may also fall under Title 18.

FDA personnel responsible for pesticide analyses also consult Title 40, "Protection of the Environment." Part 180 of 40 CFR includes the pesticide residue tolerances for foods and feeds established by the Environmental Protection Agency and enforced by FDA.

1.2.4 Federal Register

The Federal Register (FR), an official government publication, is issued daily Monday through Friday. It makes known to the public the regulations and legal notices issued by Federal agencies, as well as other Federal issuances prescribed to be published in the FR. The FR is the source for the regulations published annually in the Code of Federal Regulations. Regulations published in the FR are effective on the date stated in the FR unless "stayed," i.e., not to be put into effect for some reason. If a regulation is "stayed," the FR publishes that notice.

1.3 Analytical Methods

The analyst will use methods from a number of sources to determine regulatory compliance. These include methods described in official compendia, in manuals relating to analytical categories, and in FDA compliance manuals.

1.3.1 Official Compendia

The FD&C Act and regulations cite publications that contain methods to determine if a product conforms to legal requirements. The compendia cited have gained official status within the Federal judicial system as methods of choice. Most commonly used in the laboratory are the United States Pharmacopeia (USP), National Formulary (NF) and Official Methods of Analysis of AOAC International (formerly the Association of Official Analytical Chemists). These books are referred to here as USP, NF, and AOAC. Note that the USP and NF are now combined into one publication, and that we use the acronym AOAC to refer to both the compendia and the professional organization.

Official methods are those in compendia specified in the Food, Drug & Cosmetic Act and prescribed in the Code of Federal Regulations. Official methods are used, whenever found, or unless otherwise specified, for either original or check analysis, when regulatory actions are based on analytical findings.

Four common sources of methodology, and their citation in the Act or CFR, are listed below. Review the citations for pertinent information.

- AOAC [21CFR 2.19]
- United States Pharmacopeia (USP) [FD&C Act, 201 (g)(1), 201 (j), 501 (b)]
- Pesticide Analytical Manual (PAM) [40 CFR 180.101 (c)]

- Food Chemicals Codex [21 CFR 170.30]

Methods in manufacturer's Applications and Petitions that have been approved have "official" status. These include New Drug Applications (NDA), Abbreviated New Drug Applications (ANDA), New Animal Drug Applications (NADA), Food Additive Petitions (FAP), and Pesticide Petitions (PP).

Official methods always have higher status and are preferred over non-official methods. However, the method is only official for the product matrix and performance range specified for the method.

Non-official methods may be used for determining compliance. Method validation will be submitted when a non-official method is used for a sample recommended for regulatory action. See section ORA Lab Manual, Volume IV, Chapter 1.3.4.

1.3.2 Specialized Manuals

In addition to official compendia, FDA also relies on a large number of analytical manuals that pertain to analytical categories. These manuals contain both official and unofficial methods and are generated by FDA, other agencies, professional associations, and trade associations.

The FDA-produced manuals are a collection of the most reliable methods for program areas. They may include official and unofficial methods and be compiled from methods from other agencies, professional associations, and trade associations. Two of the most widely used analytical manuals are the Bacteriological Analytical Manual (BAM) and the Pesticide Analytical Manual (PAM). Both the BAM and PAM are found on the Internet.

1.3.3 Other Method Sources

In addition to official compendia and specialized manuals, certain methods may be directed for use by sources other than the CFR and the Act. The Compliance Program Guidance Manual, Compliance Policy Guides, Import Alerts, and special Assignments may dictate a particular method to be used. Before embarking on a long search for methodology for a particular sample be sure to check these sources (especially the compliance programs) for methods which may be specified. Choosing another method could have adverse impact on the regulatory decision.

Often unofficial methods are specified, and these commonly come from scientific journals and the Laboratory Information Bulletins (LIB). LIBs are a popular source of unofficial methodology in FDA published by the Division of Field Science, ORA. Peer-reviewed scientific journals are found for all areas of analytical science. One of the most used is the Journal of the AOAC International, which is geared toward analytical chemistry. This journal is unique in that the AOAC organization was originally sponsored by (and for) the federal government, and it was under the auspices of the FDA until its independence in 1979.

The specified methods may or may not be official and have been chosen because they have been deemed the best method for the job. Consistency in method selection is important, as more than one laboratory may perform the analysis. Proper method selection is the responsibility of the analyst, with concurrence from the supervisor.

1.3.4 Method Validation

Validation of a method is the planned and documented procedure to establish its performance characteristics. The performance characteristics or the validation parameters of the method determine the suitability for its intended use. They define what the method can do under optimized conditions of matrix solution, analyte isolation, instrument settings, and other experimental features. Method validation often includes the determination of the following: system suitability, linearity region, accuracy, precision, specificity, detection limit, ruggedness, and recovery. Comparison of the results to another method, the use of blank reagent and matrix runs, and independent runs performed by another analyst are also used.

Sometimes an alternative method source may not need validation. Examples include "non-standard" methods that a lab would not have to validate if the validation data was in existence and performed by a collaborative study or some other formal protocol which evaluated the methods performance (accuracy, precision, ruggedness, specificity, linearity, detection, etc). These methods need proof of validation from a recognized certification process such as AOAC Peer Verification or Performance Testing. Certification of method validation is essential when using test kits as an additional method source.

1.4 The Sample

A sample, according to one dictionary, is a specimen, a part, or one of a number intended to show the kind and quality of the whole. Another dictionary says a sample is a part of anything presented for inspection or shown as evidence of the quality of the whole. We can draw from these definitions the essence of what a sample is to us.

1.4.1 FDA Sample Requirements

The FDA sample is a representative portion of a product that is to be analyzed to determine something about the quality of the batch or lot sampled. To make the sample representative, certain sampling procedures are used. Examples of these can be found in the Inspectors Operations Manual (IOM) or the pertinent compliance program.

The sample serves both as analytical raw material and as physical evidence in a court of law. We seldom know whether a sample will have to serve as legal evidence, so we make the assumption,

from the beginning that it will. This means that the sample is treated with great care with respect to its identity, integrity and history.

Sample integrity is a most important concept that is to be thoroughly understood by all persons who handle samples, from the sample collector, through the sample custodian, to the physical science aide, to the analyst. When a sample is introduced into evidence, FDA establishes that it is the correct sample (identity) and that it has not been changed in any way prior to receipt by the analyst, and that it represents the state of the lot as originally sampled. To say "not to our knowledge" is not sufficient. Everyone in the chain of custody is able to prove the integrity of the sample, conclusively.

1.4.2 Sample Accountability

Before the analyst analyzes an FDA sample, the training supervisor will instruct the analyst in the policies and procedures that are to be followed to preserve sample integrity and to show continuity of sample handling. Sample accountability calls for entries on the analytical worksheet and in the electronic FACTS database to document who handled the sample and when and how sample integrity was maintained. The instructions are given in the Field Accomplishments and Compliance Tracking System (FACTS) guidelines or local Standard Operating Procedure (SOP) provided by the local FACTS trainer. Study the FACTS documents and follow the prescribed procedures when handling samples.

1.4.3 Reporting Sample Analysis

The analyst is responsible for an accurate and complete analysis of the sample and a written report of the analysis. All analytical data is to be recorded only on the FDA worksheet designed for the purpose [the Analyst Worksheet (FD-431), or modification of this form] and on supporting documents, such as instrument charts, which are attached to the worksheet – unless otherwise defined by an approved deviation in the laboratory's Quality Management Plan. Approval of any deviations is to be obtained through the Division of Field Science.

Before the analyst analyzes a sample, including samples collected solely for training purposes, the training supervisor will instruct the analyst on how to prepare a worksheet. ORA Lab Manual, Volume II, sub-section on "Analyst Records", describes the policy and gives instructions for preparing the worksheet.

1.4.4 Field Accomplishments and Compliance Tracking System (FACTS)

The Field Accomplishments and Compliance Tracking System (FACTS) was developed to centralize the data gathered by ORA into one nation-wide system. Therefore, FACTS is recognized as FDA's automated system for field assignments, analytical results, firm information, compliance actions, and time reporting. FACTS incorporates assignment management and work results for the following laboratory activities: sample accountability, analytical reporting, analyst time reporting and sample dispositions. Coupled with writing of the analytical worksheet, using FACTS will be an essential part of the analyst's responsibility to report analytical information on all work products.

Basic FACTS training will be provided by the local FACTS cadre or training unit during the training period.

1.5 Additional Records

1.5.1 Notebook

Notebooks may be kept to record data and observations that are not related to samples. During training, tabulations of assignments and reports on completed work or training sessions notebooks may be useful.

The notebook is the analyst's record of observations made during work in the laboratory. It is subject to the limitations outlined under Notebooks (see ORA Lab Manual, Volume II). Data generated during a sample analysis and related to a sample is to be recorded only on the analytical worksheet or its attachments, never on scrap paper or in the notebook. Comments on methods used may be recorded in the notebook but without reference to a sample number. Information on difficulties encountered with methods and how they were overcome should be an integral part of the analyst's training records.

1.5.2 Training Progress Report

To better evaluate the analyst's progress during training, the supervisor expects one or more reports, including:

- A. A periodic tabulation of completed analyses and other assignments (see Form 1A). This tabulation is mandatory through the basic training period.

B. An oral or written response on each exercise, analysis or training section, covering the points outlined below. (see Form 1B)

1. Analysis

- Principles of the analysis (chemical equations, metabolic process, filth isolation techniques, etc.).
- Weakness or pitfalls in the procedure used.
- Specificity of the method: what (ion, microbe, insect part, etc.) was identified and measured. Was the procedure direct or indirect?
- What other sample constituents could give the same result using this method?
- What conditions of the sample or the environment could cause higher or lower than true results?
- What controls were run concurrently with the sample?
- What other methods or procedures could be used? List their advantages and disadvantages.
- (optional) Further questions that could be discussed include: Give an example calculation of the results using significant figures and units; List the % error introduced by the instrument, dilutions, and measurements; What concentration range and matrix the procedure was designed for; How the accuracy and precision were determined; What role the analyst's technique played in the accuracy and precision; and What precautions were used for safety, waste disposal and contamination.

2. Program (for sample analyses)

- Under which compliance program should the sample have been collected? Summarize the background of that program.
- Was Part IV of the compliance program followed? Does the program prescribe that methods to be used?
- Was the FACTS data entered correctly?

3. Legal Aspects

- What is FDA's legal interest in the product? Under what part of the Act (or other law) does FDA have jurisdiction? Do the records or the Collection Report (C/R) indicate or prove interstate movement of the product?

- How is the consumer protected by our analysis of this product?
Approximately how much (estimate) did this protection cost?
- If the sample was violative, how might a defense attorney attack our analysis or handling of the sample? How can this be avoided or countered?

1.6 Other Information

Analysts also are to be familiar with proper laboratory administrative and regulatory procedures and observe the safety, waste disposal and quality assurance programs in place for their laboratories.

1.6.1 Administrative and Regulatory Procedures

The Field Management Directives (FMD), Regulatory Procedures Manual (RPM), and Compliance Policy Guides (CPG) are FDA publications that contain pertinent information such as the laboratory research directives, product recall information, and other statements of FDA regulatory procedures. These on-line manuals can be found on the FDA Intranet site and on the FDA Gold Disk.

1.6.2 Laboratory Safety

Each laboratory has its own safety and waste disposal programs; the supervisor should give copies of these. Nothing is to be put down the drain unless specified as allowable under the waste disposal program.

Read the general laboratory safety instructions found in ORA Lab Manual, Volume III, Section One, "Environmental Health and Safety," and in the laboratory's program. The analyst is to understand all instructions before beginning any training exercise.

Methods in the AOAC Official Methods of Analysis contain references to safety notes in "Appendix: Laboratory Safety," which identifies hazards and means for avoiding or protecting against them. Review instructions under Equipment and references under the method before beginning the analysis. Also consult the MSDS sheets for safety notes. Safety and cross contamination precautions are used with all chemicals. Special care is to be taken to prevent injury from toxic chemicals, explosions, fires, and corrosive chemicals. Some chemicals or organisms may demand special precautions such as use of protective clothing or use of an enclosed balance.

1.6.3 Quality Assurance

Each FDA laboratory has a quality assurance program (QAP) tailored to its individual responsibilities within headquarters imposed standards. FDA analysts are to participate in the QAP program, perhaps to analyze a national check sample or by assignment to monitor the performance of an instrument. FDA is implementing a national quality assurance program that is based on the ISO 17025 standard for testing and calibration laboratories.

1.7 General References

This section lists general reference texts that may be useful to the FDA analyst. The list is neither comprehensive nor exclusive. Other texts may be substituted and supplemental texts added. Publications on a special topic (e.g. drugs) will be included in the pertinent section of this manual. A good bibliography of basic texts on all areas of chemical and instrumental analysis is found on-line at the Journal of Chemical Education's [Chemical Education Resource Shelf](http://www.umsl.edu/~chemist/books/texts.html). (www.umsl.edu/~chemist/books/texts.html).

1.7.1 Handbooks and Indexes

1. Handbook of chemistry and physics (current ed.). Boca Raton, FL: CRC Press.
2. Lange's Handbook of chemistry. New York: McGraw-Hill.
3. The Merck index. Rahway, NJ: Merck & Co.

1.7.2 Statistics

1. Youden, W.J., Steiner, E.H. (1997). Statistical manual of the AOAC (5th printing). Arlington, VA: AOAC International.
2. Meier, P.C., Zund, R.E. (2000). Statistical methods in analytical chemistry (analytical chemistry Vol. 123, 2nd ed.). New York: John Wiley & Sons.

1.7.3 Analytical Chemistry

1. Skoog, D. A., West, D. M., Holler, F. J. (1995). Fundamentals of analytical chemistry (7th ed.). Brooks/Cole Publishing Company.
2. Pecsok, R. L., Shields, L. D., Cairns, T., Williams, I. G. (1976) Modern methods of chemical analysis (2nd ed.). New York: Wiley-Interscience.

3. Baiulescu, G. E., Stefan, R., Aboul-Enein, H. Y. (2000). *Quality and reliability in analytic chemistry* (1st ed.). Boca Raton, FL: CRC Press.
4. Harvey, D. T. (1999). *Modern analytical chemistry* (1st ed.). New York: McGraw Hill.

1.7.4 Instrumentation

1. Skoog, D. A., Holler, F. J., Nieman, T. A. (1997). *Principles of instrumental analysis*, (5th ed.). Brooks/Cole Publishing Company.
2. Settle, F. A. (Ed.). (1997). *Handbook of instrumental techniques for analytical chemistry* (1st ed.). Prentice Hall.
3. Rubinson, K. A., Rubinson, J. F. (2000). *Contemporary instrumental analysis* (1st ed.). Prentice Hall.
4. Poole, C. F., Schuette, S. A. (1984). *Contemporary practice of chromatography*. New York: Elsevier.
5. Snyder, L. R., Kirkland, J. J. (1979). *Introduction to modern liquid chromatography* (2nd ed.). New York: Wiley-Interscience.
6. Meyer, V. (1999). *Practical high-performance liquid chromatography* (3rd ed.) New York: John Wiley & Sons.
7. Jennings, W., Mittlefehldt, E., Stremple, P. (1997). *Analytical gas chromatography* (2nd ed.). Academic Press.
8. Scott, R. P. W. (1997). *Introduction to analytical gas chromatography* (2nd ed.) New York: Marcel Dekker.

1.8 Orientation Exercises

The analyst becomes familiar with laboratory apparatus and glassware, preparation of standard solutions, and proper techniques that are common to many analytical procedures.

The completion of all or only parts of the orientation exercises listed in this section is left to the discretion of local management. Many of these exercises may not be applicable to the scientific

discipline to which analysts are assigned. Therefore, these orientation exercises may be modified to meet the needs of the local laboratory.

1.8.1 Analytical Balances

A. Objectives

1. To determine how different laboratory balances function, their weight ranges, and their limitations.
2. To develop techniques for accurate weighing.
3. To determine which balances to use for a defined purpose (i.e. top-loader vs. analytical vs. microbalance).

B. Assignment

1. Determine the sensitivity at maximum load and at half maximum load of each type of balance assigned. Determine the minimum mass detected by balance(s).
2. Weigh a nickel coin on the balance, remove the coin, and re-zero the balance. Repeat this process three times. Then repeat the process using another similar balance as well as a different type of balance. How do the weights compare? Repeat the process using a 100 mL beaker.
3. Weigh five different nickels. Determine the average mass, the standard deviation (SD), the % relative standard deviation (%RSD), and the % error assuming that a standard nickel should weigh 5.000g.
4. Obtain a standard set of weights and calibrate a top-loader balance using 0.5, 1, 5, 10, 25, 50, and 100g weights. Plot the percent error against each weight tested. Are these errors within the limits set by the balance manufacturer?

C. References

1. American Society for Testing and Materials International. (2003). E 617-97 Standard Specification for Laboratory Weights and Precision Mass Standards. Retrieve from <http://www.astm.org>
2. U.S. Pharmacopoeia and National Formulary (current ed.). (General Chapters, section 41, Weights and Balances, pp. 1689-1681). Gaithersburg, MD: Association of Official Analytical Chemists International.

3. Skoog, D. A., West, D. M., Holler, F. J. (1990). Analytical chemistry an introduction (5th ed., p. 514). Philadelphia: Saunders College Publishing.
4. USP General Notices and Requirements.

D. Questions

1. Define precision. How is it related to accuracy? How does one measure precision?
2. What is the USP definition of “accurately weighed” and how does it relate to “measurement uncertainty”. How is the measurement uncertainty of a balance determined?
3. What do values of +/- 1 SD, of +/- 2 SD, and of +/- 3 SD tell us?
4. What is the relationship between SD and % RSD. What is the benefit of using %RSD?
5. Describe two possible ways in which samples could be contaminated during weighing.
6. What are some of the common errors in weighing and describe how to minimize them?

1.8.2 pH Meters

A. Objectives

To identify the principles involved in conducting an accurate potentiometric test by:

1. Studying the operating principles of a pH meter and its electrodes.
2. Examining the various electrodes and their use as well as examining other ways of determining pH.
3. Carrying out simple determinations of pH.

B. Assignment

1. Review references, SOPs and operating manuals for each type of pH meter in the laboratory.

2. Prepare and measure with a pH meter the pH of one or more of the buffers described in AOAC or USP/NF as directed by the trainer. Compare each measured pH to the theoretical value of the buffer(s). Plot buffer value versus measured pH.
3. List and describe several electrodes in the laboratory. Discuss the use and proper care of each.
4. List and describe each type of pH-indicating test paper used in the laboratory. What is the range of each? What is its accuracy? What are the storage parameters?
5. List and describe several common pH end-point indicators in the laboratory.
6. Draw and label a simplified schematic of a pH meter with reference and indicating electrodes.
7. Measure the pH of deionized water. Why is the pH reading unstable? Why is the pH reading different than 7.00?

C. References

1. AOAC official methods of analysis. (current ed.). Sections on "Buffer Solutions for Calibration of pH Equipment" and "Standard Buffers and Indicators for Colorimetric pH Comparisons" in Appendix: "Standard Solutions and Certified Reference Materials." Gaithersburg, MD: Association for Official Analytical Chemists International.
2. U.S. Pharmacopeia and National Formulary (current ed.). (General Chapters, Section 791, "pH.", "Indicator and Test Papers." and "Buffer Solutions."). Gaithersburg, MD: Association for Official Analytical Chemists International.
3. Beyon, R. J., Easterby, J. S. (1996). Buffer solutions (ISBN 0199634424). Oxford, UK: BIOS Scientific Publishers.

D. Questions

1. Calculate the pH of a 0.075 M solution of acetic acid; assume the $K_a = 1.8 \times 10^{-5}$ for acetic acid. Prepare such a solution and measure the pH, calculate the % error, and give at least three reasons for the error.
2. Would a pH measurement in MeOH or in 50% MeOH - H₂O be accurate? Why or why not?
3. Define buffer capacity.

4. List several factors involved in selecting a buffer for a given procedure.
5. What is the useful range in buffering capacity about the pKa of the weak acid?
6. Describe how to prepare a liter of 0.0100 M phosphate buffer at pH = 8.00 using only 10.0 M H₃PO₄ and a concentrated NaOH solution.
7. Discuss the use of an "equivalent weight" of an acid or base.
8. What kinds of determinations, other than pH, may be made using a pH meter? Give two examples.

1.8.3 Volumetric Glassware

A. Objectives

1. To develop good technique in the handling and use of volumetric glassware.
2. To calibrate one or more pieces of laboratory volumetric equipment, to review National Institute of Standards and Technology (NIST) and USP/NF requirements for volumetric glassware, and compare these to the calibration values obtained.

B. Assignment

1. Review references.
2. Obtain a density vs. temperature table for water and accurately calibrate each piece of volumetric glassware supplied by the trainer (e.g., volumetric pipet, volumetric flask, buret). Calculate the % error for each measurement and compare to stated accuracy.
3. Measure the delivery times of two or three pipets of assorted volumes using a stopwatch. Compare with NIST, USP, etc., standards and manufacturers' specifications.
4. Convert the volume of one of the measurements at temperature of measurement to the volume at 20°C and 25°C.
5. Be prepared to explain the complete calibration operation, including all steps in the calculation, the use of significant figures, precision, and accuracy.

C. References

1. <http://www.nist.gov/>
2. <http://ts.nist.gov/WeightsAndMeasures/resources.cfm>
3. <http://ts.nist.gov/WeightsAndMeasures/Publications/appxc.cfm>
4. Harris, D.C. (August 1998). Quantitative chemical analysis (5th. ed.).
5. U.S. pharmacopoeia 25 and national formulary 20. (General Chapter, Section 31, "Volumetric Apparatus."). Taunton, MA: Rand McNally.
6. Leveson, D. J. (2002). Rounding of Numbers, How, When and Why. Retrieve version from <http://academic.brooklyn.cuny.edu/geology/leveson/core/linksa/roundoff.html>

D. Questions

1. Can all volumetric glassware be NIST certified?
2. What do the letters "TC" and "TD" signify?
3. Why should freshly boiled water be used in calibrating glassware?
4. How would someone know when a piece of volumetric glassware is not clean? What method would be used to clean it?
5. List the manufacturer's % accuracy of the following types of volumetric equipment: Class A 10 mL pipette, Class A 100 mL volumetric flask, 10 mL Mohr pipette, 1 mL Auto Pipette, 10 uL Auto Pipette, 100 mL graduated cylinder, 250 mL graduated beaker.
6. List several of the variables involved in correctly using a 10 mL volumetric pipette.

1.8.4 Standard Solutions

A. Objectives

To identify the principles and techniques involved in conducting a successful, accurate standardization by:

1. Using one or more official methods for standardization of volumetric solutions.
2. Preparing one or more standard laboratory solutions for later use or for other analysts.
3. Preparing an accurate dilution of a standard solution.

B. Assignment

1. Review references.
2. Prepare a solution of 0.1 N NaOH, 0.1 N HCl, or other solutions as assigned by the trainer. Using an official method (AOAC or USP/NF), standardize the solution. Perform the analysis in triplicate and calculate the %RSD.
3. Prepare 100 mL of 0.01N NaOH from the standardized 0.1N NaOH to within 1% accuracy.

C. References

1. AOAC official methods of analysis. (current ed.). (Appendix: "Standard Solutions and Certified Reference Materials."). Gaithersburg, MD: Association of Official Analytical Chemist International.
2. U.S. Pharmacopeia 25 and National Formulary 20 (current ed.). (General Chapters, Section on "Volumetric Solutions."). Taunton, MA: Rand McNally.
3. Volumetric Solutions: Preparation and Standardization
 - General Information: http://pubs.acs.org/reagents/reagent2000/sec_e000.html
 - Preparation and Standardization: http://pubs.acs.org/reagents/reagent2000/sec_e001.html

D. Questions

1. What is a primary standard? What are its essential properties? Why was the standard dried?
2. What conditions are to be met to maximize the accuracy of a standardization?
3. Define the normality factor and discuss its use.
4. Why couldn't one accurately weigh out solid NaOH to make the 0.1 N standard NaOH solution?
5. Define a "1 in 2" solution; a "(1 + 2)" solution; a 10% W/W KI solution; a 10% V/V methanol - water solution; a 5% W/V acetic acid solution; a saturated solution. Calculate the normality and molarity of a 10% H₂SO₄ solution.

1.8.5 Thermometers

This exercise is to be modified for different analytical disciplines and may be integrated with other exercises.

A. Objectives

1. To familiarize the analyst with different types of liquid-in-glass thermometers.
2. To establish the proper use of each type of thermometer.
3. To familiarize the analyst with the methods used to calibrate thermometers.

B. Assignment

1. Review references for care, handling, and calibration of liquid-in-glass thermometers.
2. Calibrate one each of the following (if the laboratory uses them): partial immersion, total immersion, complete immersion, and maximum recording thermometers using two standards for each thermometer.

C. References

1. American Society for Testing and Materials International. (1984, March). *E 77-84 Standard* (pp. 59 – 72).

2. Wise, J. A. NIST Special Publication 819, A procedure for the Effective Recalibration of Liquid-in-Glass Thermometers.

D. Questions

1. Define partial immersion, total immersion, and complete immersion as related to thermometers.
2. How can an analyst recognize the different types (partial, total, or complete) of immersion thermometers?
3. How does a maximum recording thermometer differ from a complete immersion thermometer?
4. What is the best method for reuniting a separated mercury column?
5. Why should an open flame not be applied to a thermometer to raise the temperature?
6. Why are two or more standards generally used to calibrate a thermometer?
7. Why is the boiling point of pure H₂O generally not used as one of the two calibrating standards?
8. How many significant figures can be obtained from the temperature reading of a normal thermometer at room temperature?
9. List another typical device for measuring the temperature besides a glass thermometer.

1.8.6 Microscopes

This exercise is to be modified for different analytical disciplines and may be integrated with other exercises.

A. Objective

1. To acquaint the analyst with the proper care and use of the light microscope, i.e.:
 - Study the operation of the microscope parts.
 - Learn the correct procedures for cleaning the microscope.
 - Learn the procedure for light alignment of the microscope.

- Learn the method for determining the microscope factor of the microscope.
- Learn the method of calibrating an ocular micrometer.

B. Assignment

1. Review reference material found in the lab on the microscope.
2. Clean the objectives, condenser, and oculars.
3. Properly align the microscope.
4. Determine the microscope factor of the microscope. Prepare a slide and determine the microscopic count of a food sample.
5. Calibrate an ocular micrometer. Measure the length and width of some stained bacteria.

C. References

1. (1998). Bailey and Scott's diagnostic microbiology (10th ed., chap. 11, pp.134-150). St. Louis, MO: Mosby Co.
2. Goldstein, D. J. (1999). Understanding the light microscope, a computer-aided introduction (1st ed.). Academic press.
3. Manuselis, G., Mahon, C. R. (2000). Textbook of diagnostic microbiology (2nd ed.). AACC press.
4. Burrells, W. (1977). Microscope technique: a comprehensive handbook for general and applied microscopy. New York: John Wiley & Sons.
5. Murphy, D. B. (2001). Fundamentals of light microscopy and electronic imaging (1st ed.). Wiley-Liss.
6. American Public Health Association. Standard methods for examination of dairy products. (current ed.). Washington, DC: American Public Health Association.
7. Murray, P. (1995). Manual of clinical microbiology (6th ed., chap. 4) Washington, DC : American Society of Microbiology Press.

D. Questions

1. Define the following types of illumination: bright field, dark ground, phase contrast, and fluorescence.
2. Where is each form of illumination used?
3. What are the two ways someone can tell an oil immersion lens from a high dry lens?
4. List and describe each objective in the nosepiece.
5. List and describe the ways the light source may be used to gain the best resolution.
6. List and describe the cleaning procedure for each microscope used in the laboratory.

1.9 Appendix -- Answer Key

1.8.1 Balances

1. Define precision. How is it related to accuracy? How does one measure precision?

Precision is a measure of how closely the measured values are grouped. Measurements with good precision have a tight or close grouping. Accuracy, on the other hand, is a measure of how closely the average value of the measurements is to the true value. Ideally we strive for good accuracy and good precision. It is possible to have poor accuracy and good precision, good accuracy and poor precision (luck), and poor accuracy and poor precision. We usually use standard deviation or a derivative of standard deviation to measure precision.

$$SD = \left[\sum (X - X_i)^2 / n - 1 \right]^{1/2}$$

Where X = Average Value
 Xi = Individual Value
 n = Number of Runs

2. What is the USP definition of “accurately weighed” and how does it relate to “measurement uncertainty”. How is the measurement uncertainty of a balance determined?

Accurate weighing is to be performed with a weighing device whose measurement uncertainty does not exceed 0.1% of the reading. We can determine the accuracy of a balance by comparing the balance reading with the stated value of the standard mass used.

Measurement uncertainty is the combination of random and systematic error. Measurement

uncertainty is satisfactory if three times the standard deviation (of not less than ten replicate weighings) divided by the amount weighed, does not exceed 0.001.

3. What do values of +/- 1 SD, of +/- 2 SD, and of +/- 3 SD tell us?

Measurements with a precision range of +/- 1 SD tells us that if the analyst continues to perform the measurements using exactly the same technique, then the results will fall within this range 68% of the time. +/- 2 SD = 95 % and +/- 3 SD = 98 %.

4. What is the relationship between SD and %RSD? What is the benefit of using %RSD?

$\%RSD = [SD / X] \times 100 \%$ where X is the average value. One benefit of %RSD is that the precision is normalized to a 100 point scale which allows us to readily compare precisions of two methods or analysts regardless of the analysis concentration range.

5. Describe two possible ways in which samples could be contaminated during weighing.

There are several possible contamination routes during weighing. An analyst could contaminate a sample during weighing by placing a contaminated spatula into the sample, by placing the sample on or into a contaminated holder during weighing, by dropping some lint/hair/skin or sneeze into the sample while weighing, or by opening up a bottle of chemicals near the sample being weighed. When performing trace analysis, it is possible for just a microgram of contaminant to be important, and a microgram is about 100 times less massive than a fingerprint!

6. What are some of the common errors in weighing and describe how to minimize them?

There are a number of possible ways that an error in weighing can occur. A few potential errors are:

- Misreading of the balance,
- Balance not level,
- Not cleaning the surface of the balance first,
- Touching the weighed object with moist hands,
- Leaving the balance doors open during weighing,
- Using a miscalibrated balance,
- Not cooling the sample down to near room temperature,
- Not removing a static charge from the sample,
- Excess vibration or air currents from people or nearby equipment, and
- Prolonged time sample left on pan adds/loses moisture.

1.8.2 pH Meters

1. Calculate the pH of a 0.075 M solution of acetic acid; assume the $K_a = 1.8 \times 10^{-5}$ for acetic acid. Prepare such a solution and measure the pH; calculate the % error and give at least three reasons for the error.

Let HA = Acetic Acid. $HA \leftrightarrow H^+ + A^- \quad 1.8 \times 10^{-5} = [H^+][A^-]/[HA]$

Let $X = \underline{M}$ of HA that dissociates at equilibrium.

$[H^+] = [A^-] = X \quad [HA] = 0.075 - X \sim 0.075$

$1.8 \times 10^{-5} = X^2/0.075; \quad X = [H^+] = 1.2 \times 10^{-3} \underline{M}; \quad pH = -\log [1.2 \times 10^{-3}] = 2.9$

Possible reasons for error are: a) Errors involved in making the 0.075 M acetic acid solution from concentrated acetic acid. b) The concentrated acetic acid may not be 100 % acetic acid. c) The pH meter was not calibrated properly. d) Equilibrium calculations have at least a 10%

error due to the use of **M** instead of activity; activity depends upon ionic strength, concentration, and temperature.

2. Would a pH measurement in MeOH or in 50% MeOH - H₂O be accurate? Why or why not?

No, the pH measurements would not be accurate. The surface of the indicator electrode needs to be coated with H₂O in order to function properly. Also the % ionization of the acid is dependent upon the water content.

3. Define buffer capacity.

A buffer is a solution of a weak acid or a weak base and its salt which resists a change in pH. The capacity of the buffer to resist a change in pH when either a strong acid or a strong base is added is a measure of the buffer capacity. One definition of buffer capacity is: the amount of a given acid or base that can be added to the buffer solution in order to change the pH by one unit.

4. List several factors involved in selecting a buffer for a given procedure.

The main factor is the pH -- a buffer only works if the pH is within one unit of the pK of the weak acid or weak base -- so select a buffer that has a pK as close to the pH as possible. Another factor is buffer solubility: for example, phosphate buffers may not be soluble in aqueous acetonitrile solutions. Other factors relate to buffer cost, buffer toxicity, buffer influence on microorganism growth, etc.

5. What is the useful range in buffering capacity about the pKa of the weak acid?

$\text{pH} = \text{pKa} \pm 1.0$ A buffer only works if the pH is within one unit of the pKa of the weak acid.

6. Describe how one would prepare a liter of 0.0100 M phosphate buffer at pH = 8.00 using only 10.0 M H₃PO₄ and a concentrated NaOH solution.

Add 1.00 mL of the 10.0 **M** H₃PO₄ to a little less than 1.00 L of water. Add enough concentrated NaOH to bring the pH to 8.00 (use calibrated pH meter) and then bring the volume to 1.00 L with water.

7. Discuss the use of an "equivalent weight" of an acid or base.

The equivalent weight is the mass of the acid or base that will neutralize one mole of either H⁺ or OH⁻. The use of equivalent weight, equivalents, and the corresponding Normality (**N**) is useful since one equivalent of a substance will react with one equivalent of another substance; this simplifies calculations since the balancing coefficients of the reaction do not

enter into the calculation. The use of equivalent weight, equivalents, and the corresponding Normality (**N**), is an older concept and is frequently not taught in college chemistry classes.

8. What kinds of determinations, other than pH, may be made using a pH meter? Give two examples.

One can use a pH meter as a potentiometer and measure the voltage developed at the electrodes in an oxidation-reduction reaction. Also, the pH meter can be used to measure the concentration of a given ion using an ion dedicated electrode.

1.8.3 Volumetric Glassware

1. Can all volumetric glassware be NIST certified?

All volumetric glassware can be calibrated in the laboratory using accurately weighed deliveries of water since mass measurements can be more accurate than volume measurements (a chart of the densities of water at various temperatures is needed).

2. What do the letters "TC" and "TD" signify?

The letters "TD" means "to deliver;" many volumetric pipets are calibrated to deliver a given volume after a given drain time. "TC" means "to contain;" many volumetric flasks are calibrated to contain a given volume.

3. Why should freshly boiled water be used in calibrating glassware?

The density of the water does slightly depend upon the amount of dissolved gases, and boiling is a simple way to remove dissolved gases.

4. How would one know when a piece of volumetric glassware is not clean? What method would one use to clean it?

The most common way to determine the cleanliness of volumetric glassware is to drain water from the apparatus; if the water drains smoothly and does not bead up, then it is considered clean. Volumetric glassware can become "dirty" just by picking up surface organics from the air. One way to clean the glassware is to rinse the glassware with chromic acid. Chromic acid is a solution of potassium dichromate in aqueous sulfuric acid. This solution is toxic, corrosive, and a strong oxidizing agent (caution); it will oxidize and dissolve most organics present. Other methods include rinsing with strong acid or base (depending on nature of deposit), rinsing with an organic solvent, or good old-fashioned scrubbing followed by a thorough rinse.

5. List the manufactures % accuracy of the following types of volumetric equipment: Class A 10 mL pipette, Class A 100 mL volumetric flask, 10 mL Mohr pipette, 1 mL Auto Pipette, 10 uL Auto Pipette, 100 mL graduated cylinder, 250 mL graduated beaker.

Note: The manufacturers' tolerances for Class A glassware may be better than the required standard. Class A volumetric glassware are those whose capacity tolerances are accepted by the National Bureau of Standards, and these tolerances are listed in the USP under Volumetric Apparatus [31]. The actual answers depend upon the manufacturer of the equipment; some possible answers are: Class A 10 mL pipet = 0.02 mL; Class A 100 mL Volumetric Flask = 0.08 mL; 10 mL Mohr pipet = 0.04 mL; 1 mL Auto Pipet = 0.02 mL; 10 uL Auto Pipet = 0.5 uL; 100 mL Graduated Cylinder = 0.5 mL; 250 Graduated Beaker = 10 mL.

6. List several of the variables involved in correctly using a 10 mL volumetric pipette.

Some variables involved in using a 10 mL volumetric pipet are: drain time; possible beads on the inner surface due to uncleanliness; temperature; bringing meniscus to the proper level; angle of drain; touching off last drop; rinsing of the pipet with the solution used; pipet calibration; etc.

1.8.4 Standard Solutions

1. What is a primary standard? What are its essential properties? Why does one dry the standard?

A primary standard is one which is weighed out and the concentration is known to be better than 1% accuracy. Some essential properties of the primary standard are: having a high purity; having a known purity; being stable under normal temperature and normal drying conditions; being stable under long storage conditions; and not being hygroscopic. Drying of the primary standard may be needed to bring it to a constant mass by removing any surface moisture.

2. What conditions are to be met to maximize the accuracy of a standardization?

The standards are to be of desired and known concentrations; the standard reacts quickly and completely with the reagents of interest; the instruments and glassware used allow the desired accuracy; the techniques used by the analyst are to be reproducible and allow the desired accuracy; and preferably, the weight of standard substance and volume of standard solution should not be too small. In general, titrations are made directly to the end point. A back-titration with another standardized solution increases the possibility of error. The standardization of a solution against another standardized liquid should be avoided. Every standardization should be based on at least three parallel determinations.

3. Define the normality factor and discuss its use.

Acid yields one, two, or three protons per formula. If the same number of formula weights of each, per liter, were dissolved the neutralizing capacities would not be the same. To express concentration in the terms of neutralizing capacity one does so according to the number of H_3O^+ or OH^- in the solution. A solution with one mole H_3O^+ or OH^- present for neutralization reactions is called "one normal solution." That weight of a compound or substance which will supply one mole H_3O^+ or OH^- for a neutralization reaction is called equivalent weight of the material. The equivalent weight of an acid is the formula weight divided by the number of protons per formula present for neutralization reaction.

4. Why couldn't one accurately weigh out solid NaOH to make the 0.1 N standard NaOH solution?

One can purchase a high and known purity NaOH product; so, why can't we just weigh out 4.00 g of 100% NaOH and dissolve to 1.00 L in order to make 1.00 L of a 0.100 N solution? The reason why this is not possible is that NaOH is highly hygroscopic; NaOH will rapidly absorb up to 100% of its mass in water from the atmosphere. Even if one has a pure sample of NaOH it will pick up significant amounts of moisture during a 30 second weighing.

5. Define a "1 in 2" solution; a "(1 + 2)" solution; a 10% W/W KI solution; a 10% V/V methanol - water solution; a 5% W/V acetic acid solution; a saturated solution. Calculate the normality and molarity of a 10% H_2SO_4 solution.

1 in 2 solution = 1 volume liquid A + 1 volume liquid B (or sufficient volume of liquid B to make the volume of the finished solution two parts by volume). A *1 + 2 solution* = 1 volume of chemical A and 2 volumes of chemical B. A *10% W/W KI solution* = 10 g of KI added to 90 g of water. A *10% V/V methanol - water solution* = 10 mL methanol + enough water to make 100 mL total. A *5% W/V acetic acid solution* = 5 g of 100% acetic acid added to enough water to make 100 mL. A *saturated solution* = a solution containing the maximum amount of solute in solution, the undissolved solute in equilibrium with the solution.

M of a 10% H_2SO_4 (W/V) solution: $(10\text{g H}_2\text{SO}_4 / 100\text{ mL solution}) \times (1.0\text{ mole H}_2\text{SO}_4 / 98\text{ g H}_2\text{SO}_4) \times (1000\text{ mL} / 1.0\text{ L}) = \mathbf{1.0\text{ mole H}_2\text{SO}_4 / L}$. N of a 10% (W/V) H_2SO_4 solution: for H_2SO_4 , N = M x 2 = **2.0 equivalents $\text{H}_2\text{SO}_4 / L$**

1.8.5 Thermometers

1. Define partial immersion, total immersion, and complete immersion as related to thermometers.

A partial immersion thermometer has just its lower portion - the mercury bulb up to the marked immersion line - immersed in the sample. A total immersion thermometer involves immersion of the thermometer to the top of the mercury column, with the remainder of the stem and the upper expansion chamber exposed to ambient temperature. A complete immersion thermometer is entirely immersed in the sample.

2. How can an analyst recognize the different types (partial, total, or complete) of immersion thermometers?

The partial immersion thermometer is by far, the most common, and it will usually have an immersion line etched into it just above the mercury bulb; assume any unmarked thermometer will be a partial immersion type. The other two types will be plainly marked.

3. How does a maximum recording thermometer differ from a complete immersion thermometer?

A maximum recording thermometer only indicates the highest temperature achieved.

4. What is the best method for reuniting a separated mercury column?

The best way to reunite a separated mercury column is to place the thermometer into a very cold environment -- liquid N₂ or dry ice in acetone; allow the mercury to unite in the bulb; and then slowly warm back up to room temperature.

5. Why should an open flame not be applied to a thermometer to raise the temperature?

The intense heat from the flame will expand the thermometer rapidly and possibly crack the glass.

6. Why are two or more standards generally used to calibrate a thermometer?

If one calibrates a thermometer at a single temperature, then it will be accurate at that temperature; however, it could easily be off at other temperatures - especially at temperatures far from the calibration point. To help ensure that the thermometer is accurate over a selected range of temperatures, then one calibrates the thermometer at the lower portion of the range as well as at the upper portion of the range.

7. Why is the boiling point of pure H₂O generally not used as one of the two calibrating standards?

The boiling point of water depends upon the external pressure. For example, the BP of water at 760 Torr is 100 °C, and the BP of water at 630 Torr is 94 °C.

8. How many significant figures can be obtained from the temperature reading of a normal thermometer at room temperature?

At a temperature of about 20 °C, a typical thermometer can be interpolated to 0.2 °C; so, three significant figures are possible.

9. List another typical device for measuring the temperature besides a glass thermometer.

A thermocouple is a common temperature probe. It measures the temperature sensitive electric current flow through two dissimilar metals in contact with each other and converts the current to temperature.

1.8.6 Microscopes

1. Define the following types of illumination: bright field, dark ground, phase contrast, and fluorescence.

Using bright field, object is illuminated with light. Using dark field, object reflects the light out of its surface and appears bright against a black background. In phase contrast microscopy, the unstained material can be viewed when light passes and is partially deflected by different thicknesses of the object. Fluorescence microscopy uses certain dyes. When the dye is excited by light of high energy (short wavelength UV), it emits the absorbed energy later as visible long wave light and the stained object is thus illuminated against a dark background.

2. Where is each form of illumination used?

Bright field is used with stained objects and fresh material. Dark field is used with thin motile objects. Phase contrast is used with unstained material. Fluorescence is used for special diagnostic tasks like antibody-antigen diagnostic reactions.

3. What are the two ways one can tell an oil immersion lens from a high dry lens?

It is marked 100X oil immersion lens. Its tip moves up and down.

4. List and describe each objective in the nosepiece.

Framework: basic frame work includes arm and base.

Lens system:

- a) Oculars: Two eyepieces, for the examiner to use to view object,
- b) the objectives usually three 10X, 45X and 100X, and

c) the condenser located under the stage, it collects and directs the light.
Nosepiece: to carry the lens system.
Light source: usually in the base of most microscopes.
Stage: horizontal platform that supports the microscope slide.

5. List and describe the ways the light source may be used to gain the best resolution.
 - a) Change the wavelength of the light source by using filters. A blue filter should be used. This gives short wavelength and high resolution.
 - b) Keep the condenser at its highest position, this allows maximum amount of light to enter the objective.
 - c) Keep the diaphragm up, this increase the numerical aperture. (d) Use immersion oil with the 100 X lens.
6. List and describe the cleaning procedure for each microscope used in the laboratory.

To be answered by individual instructor as requested.

1.10 Document/Change History

Version 1.2 Revision Approved: 06-06-08 Author: LMEB Approver: LMEB

Version 1.2 changes:

- 1.6.2 second paragraph, changed Volume III, Section Two to Section One.
- 1.8.1 C. Reference 1., updated website
- 1.8.3 C. References 2. and 3., updated websites
- 1.8.3 C. References 4 deleted

APPENDIX -- FORM 1A (or local equivalent; is recommended for use, but is optional)
TRAINING PROGRESS REPORT (version 3/9/2002)

NAME _____ **LABORATORY** _____

Sample Number of Exercise	Product and Type of Examination	Trainer	Date assigned	Date Completed	hours

APPENDIX -- FORM 1B (Required)
TRAINING EXERCISE REVIEW AND COMPLETION (version 3/9/2002)
 Use for ISO 17025 Documentation and Bingo Card Entry

NAME:	PERFORMANCE LEVEL RATING Performance Level Rating (1) Acceptable, (2) Accepted with comment (3) Unacceptable For each factor rated other than "1", show on the front and/or back of this form: (A) Specific reason for the rating, (B) Corrective action taken or planned and (C) Analyst's comments	
EXERCISE (e.g. sample number, product, reference)		
	Performance Level Rating	COMMENTS
1. Assignment performed in a logical and orderly manner		
2. Good technical skills used, e.g. proper handling of equipment, samples, reagents and standards, correct transfer and dilution techniques, avoidance of sample contamination		
3. Method followed or deviations are reasonable and are recorded on worksheet.		
4. Raw data recorded only on the worksheet at the time obtained. It is identified immediately and completely.		
5. Samples, subsamples, solutions, reagents and media are identified.		
6. Correct equipment, glassware, solvents, media and reagents used.		
7. Security measures and safety precautions are taken.		

	Signature/date
TRAINEE: I verify that I have successfully completed the training identified above, and have completed a training sample pertaining to this analysis.	
TRAINER: I verify that the trainee has received the training identified in the objective, and has demonstrated the ability to independently perform this analysis	
SUPERVISOR: I certify that the trainee has received the training identified above and has demonstrated the ability to perform this analysis	