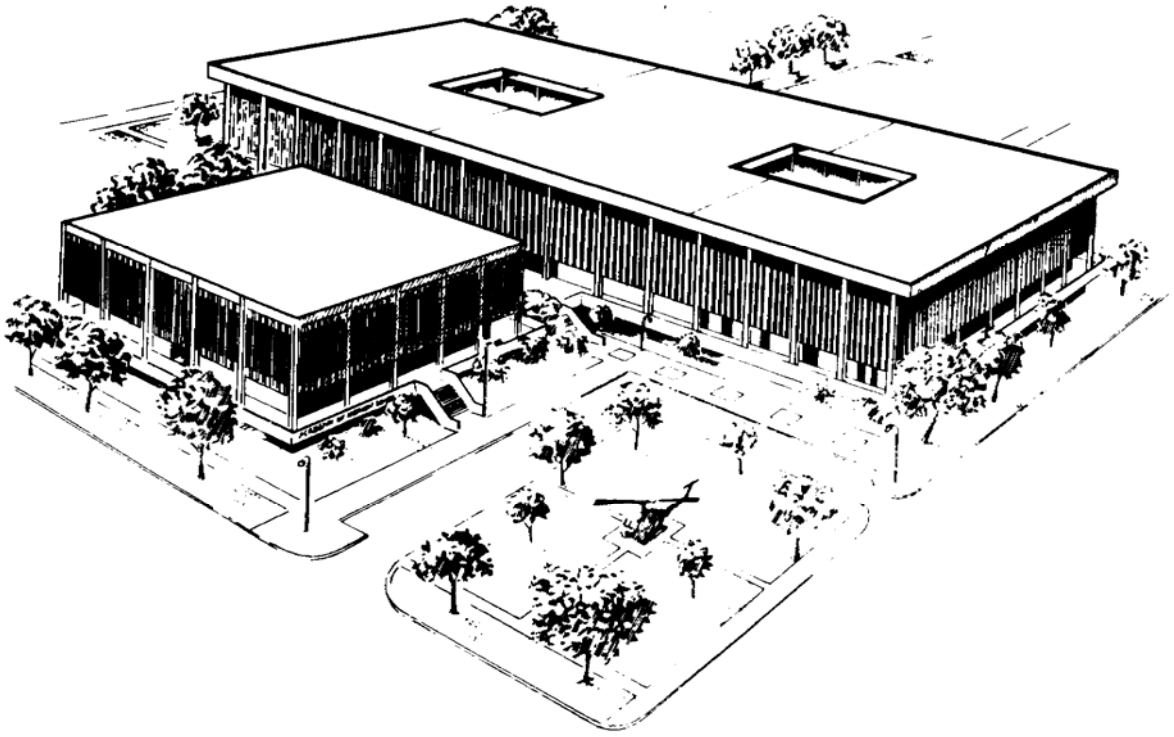
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6100



# CLINICAL CHEMISTRY I

## SUBCOURSE MD0861 EDITION 200

## DEVELOPMENT

This subcourse is approved for resident and correspondence course instruction. It reflects the current thought of the Academy of Health Sciences and conforms to printed Department of the Army doctrine as closely as currently possible. Development and progress render such doctrine continuously subject to change.

## ADMINISTRATION

For comments or questions regarding enrollment, student records, or shipments, contact the Nonresident Instruction Section at DSN 471-5877, commercial (210) 221-5877, toll-free 1-800-344-2380; fax: 210-221-4012 or DSN 471-4012, e-mail accp@amedd.army.mil, or write to:

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Approved students whose enrollments remain in good standing may apply to the Nonresident Instruction Section for subsequent courses by telephone, letter, or e-mail.

Be sure your social security number is on all correspondence sent to the Academy of Health Sciences.

## CLARIFICATION OF TRAINING LITERATURE TERMINOLOGY

When used in this publication, words such as "he," "him," "his," and "men" are intended to include both the masculine and feminine genders, unless specifically stated otherwise or when obvious in context.

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## USE OF PROPRIETARY NAMES

The initial letters of the names of some products are capitalized in this subcourse. Such names are proprietary names, that is, brand names or trademarks. Proprietary names have been used in this subcourse only to make it a more effective learning aid. The use of any name, proprietary or otherwise, should not be interpreted as an endorsement, deprecation, or criticism of a product; nor should such use be considered to interpret the validity of proprietary rights in a name, whether it is registered or not.

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**CORRESPONDENCE COURSE OF**
**THE U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL**

**SUBCOURSE MD0861**

# CLINICAL CHEMISTRY I

## INTRODUCTION

Clinical chemistry is a very dynamic field of science. Current knowledge in the field is reflected in the next two subcourses you are about to study. Subcourses MD0861 and MD0863, Clinical Chemistry I and II, address areas of particular importance in clinical chemistry and toxicology I don't.

Subcourse MD0861, Clinical Chemistry I, provides you with a background in the laboratory basics of clinical chemistry. Laboratory safety; collection, preservation, and shipment of specimens; measurement of weights and volumes; introduction to quality control; and introduction to organic chemistry are presented in this subcourse.

It is necessary for you to master the content of this subcourse before proceeding to the next one. Subcourse MD0863 will cover the major biological macromolecules of carbohydrates, lipids and proteins.

As you begin your study/review in these clinical chemistry subcourses, you are encouraged to read and review other sources of information in regard to clinical chemistry. Such self-directed learning efforts on your part will provide you with skills to continue your learning long after you complete this subcourse series. Furthermore, as you know, the amount of knowledge in clinical chemistry will not be static. Therefore, you must continue to read and study material related to the area in order to remain current in your knowledge.

**Subcourse Components**:

The subcourse instructional material consists of five lessons as follows:

Lesson 1, Laboratory Safety.
Lesson 2, Collection, Preservation, and Shipment of Specimens.
Lesson 3, Measurement of Weights and Volumes.
Lesson 4, Introduction to Quality Control.
Lesson 5, Introduction to Organic Chemistry.

**Credit Awarded**:

Upon successful completion of the examination for this subcourse, you will be awarded 8 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Section at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

## LESSON ASSIGNMENT

**LESSON 1** Laboratory Safety.

**TEXT ASSIGNMENT** Paragraphs 1-1 through 1-6.

**LESSON OBJECTIVES** After completing this lesson, you should be able to:

1-1. Select the elements of an effective laboratory safety program and the responsibilities of the laboratory safety NCO or supervisor.

1-2. Select the statement which best describes the function of hazard warning signs commonly used in the laboratory.

1-3. Select the appropriate labeling of a National Fire Protection Association Hazardous Material warning sign that corresponds with the specific chemical or chemical reaction.

1-4. Select the statement that best describes the purpose and use of the Material Safety Data Sheet and the appropriate information it is to contain.

1-5. Select the statement which best describes the location of data on the Material Safety Data Sheet that will provide the technician with the required information or appropriate action if the property, hazard, or situation is given.

1-6. Select the statement which best describes appropriate safety considerations for work areas.

1-7. Define volatile flammables.

1-8. Select the statement which best describes how volatile flammables are to be stored and handled.

1-9. Select the statement which best describes what actions are to be taken in case of fire.

1-10. Select the statement which best describes the proper storage of chemicals, preparation of solutions, and cleanup of spills.

1-11. Select the statement which best describes the precautions to be taken when working with mercury or azides or other hazardous materials.

1-12. Select those safety considerations required when working with gas cylinders, radioactive material, and biological specimens.

1-13. Select the safety actions or precautions to be taken when working with glassware and electrical equipment.

**SUGGESTION**

After studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

# LESSON 1

# LABORATORY SAFETY

## Section I. SAFETY PRINCIPLES

### 1-1. INTRODUCTION

The clinical laboratory exposes medical laboratory specialists to a variety of potential health and safety hazards. Knowledge of these potential dangers and the precautions required to prevent accidents is essential to all involved. In the past, use of common sense was the primary form of prevention against unnecessary accidents and exposure to hazardous or infectious material. Today, safety has been emphasized through the implementation of regulations proposed by the Occupational Safety and Health Administration (OSHA). These regulations specify safety standards and equipment required by each laboratory. Other government agencies and local authorities may require additional safety standards to be met.

### 1-2. SAFETY PROGRAM

Each clinical laboratory is required to have a formal safety program. An individual is appointed as the safety officer/non-commissioned officer (NCO) to administer the program and keep it current, to investigate all accidents, and to implement corrective action to prevent its reoccurrence.

a. **Education**. All personnel, as part of their orientation to the clinical laboratory, are required to read and understand the laboratory's safety standing operating procedures (SOP). The SOP is one of the most important items in the laboratory. It is to be current, thorough, complete, and cover general and special safety practices and precautions including the special handling of toxic, hazardous, or infectious materials. It is to be kept current. Each person should be familiar with the laboratory layout and the location of emergency exits as noted in the SOP. Discussion of the location, use, and operation of fire extinguishers, fire blanket, emergency shower, eye wash, respirator, and spill kits are required. Special and standard emergency equipment are to be explained in the SOP. Periodically, discussion of safety topics should be included in the laboratory's continuing education program. Practice drills need to be conducted to remain current with procedures.

b**. Inspection**. A successful safety program is not limited to the education of the laboratory personnel. It also must include periodic inspections of the laboratory environment and equipment. Attention should be given to inspection (weekly/monthly) of the safety equipment for their proper operation, quantity, and location. All chemicals are to be checked for proper labeling and storage in approved cabinets. Electrical equipment should be checked for proper grounding. Disposal of hazardous or infectious materials should be checked for compliance with OSHA or local regulations.

c. **Warning Signs.** The identification of hazards and their location can be easily accomplished by the placement of appropriate warning signs. A variety of warning signs are available to identify the type of hazard present (see figure 1-1). The most commonly used hazard system in chemistry is the system prepared by the National Fire Protection Association (NFPA) (see figure 1-2).


Figure 1-1. Hazardous warning signs.

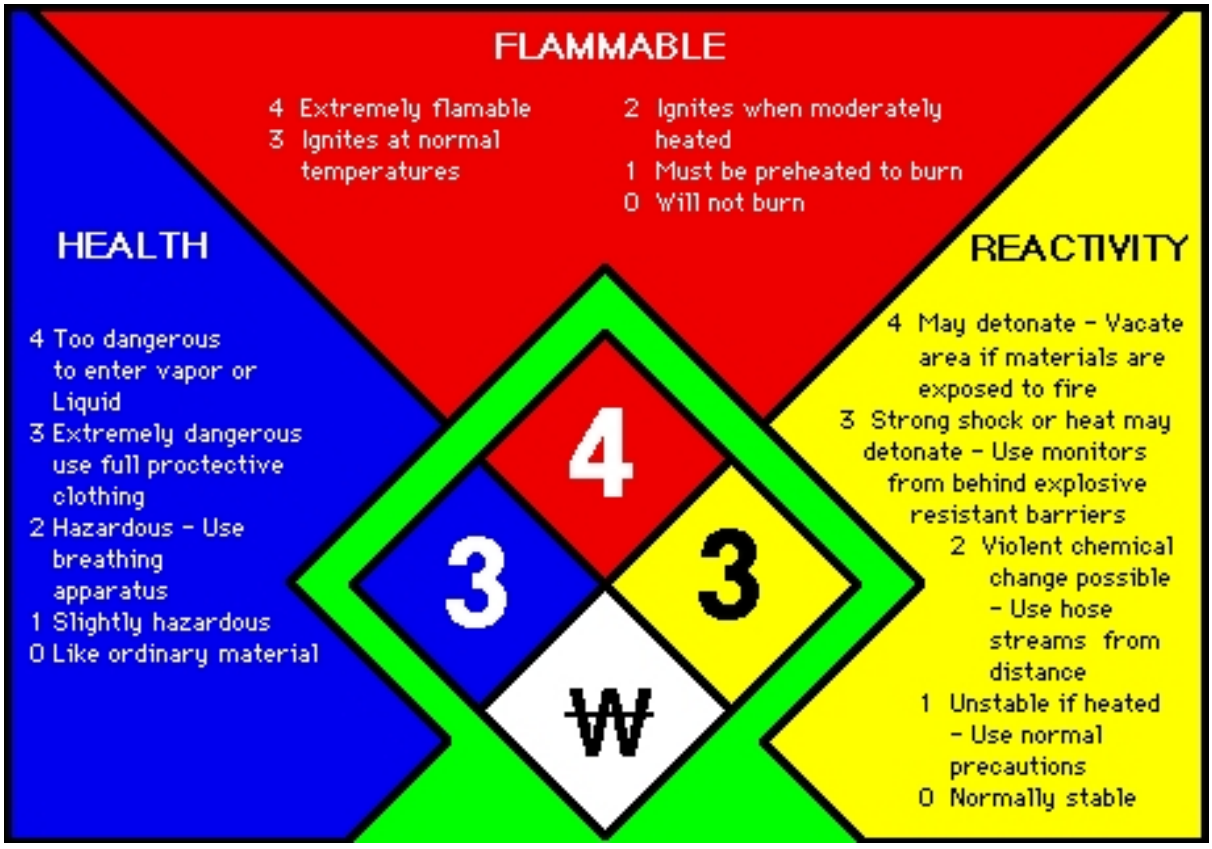


Figure 1-2. NFPA hazardous material identification system.

(1) The blue (left) diamond in figure 1-2 identifies health hazards using a 0-4 scale, 4 being reserved for the most hazardous material.

(2) The red (top) diamond identifies the degree of flammability on a 0-4 scale, 4 being a material that is extremely flammable (will ignite at temperatures below 73° F).

(3) The yellow (right) diamond identifies the reactivity or instability of the hazardous material on a 0-4 scale, 4 being the most reactive material.

(4) The white (bottom) diamond identifies special hazard information for firemen and laboratory personnel.

## 1-3. MATERIAL SAFETY DATA SHEETS (MSDS)

Material Safety Data Sheets (MSDS) provide workers and emergency personnel with ways for handling and working with a hazardous substance and other health and safety information. They will include information such as toxicity, health effects, first aid, reactivity, storage, disposal, spill/leak procedures, protective equipment and physical data (such as flash point, boiling point, etc.). MSDSs are required under OSHA's Hazard Communication and Process Safety Management Standards, EPA's Right-to-Know regulations, Department of Transportation (DOT) regulations, and other federal and state regulations. They can be obtained from the manufacturer or distributor of supplied chemicals or through the Internet. Any MSDS should be as closely matched with the hazardous substance (such as by name, lot number, serial number, etc.) as possible. The standard format for the MSDS is 16 sections. Figure 1-3 shows an example of a MSDS.

a. Section 1 gives details of the company issuing the data sheet.

b. Section 2 summarizes the major hazards associated with use of the chemical, identifies the material, and gives the CAS (Chemical Abstracts Service) and other registry numbers.

c. Section 3 identifies the material, and gives the CAS (Chemical Abstracts Service) and other registry numbers.

d. Section 4 outlines first aid measures to be followed in case of an injury caused by the product.

e. Section 5 covers fire fighting and protective equipment.

f. Section 6 outlines the procedures to be followed in case of accidental release of the chemical, including methods to be used to clean up spills. Note that these measures are unlikely to be sufficiently detailed if the chemical is particularly hazardous, and local procedures should be established to supplement what is provided in the MSDS sheet.

g. Section 7 is self-explanatory. This is an important section, sometimes overlooked by those using chemicals in the laboratory. It contains information about the possible formation of peroxides in storage, flammability, explosive risks, etc. Pay particular attention to the possible need for flammable storage cabinets, explosion-proof refrigerators, and also the need to avoid storage near incompatible chemicals.

h. Section 8 provides information on regulatory standards for exposure. In other words, the maximum permitted concentration of the material in the environment to which you are allowed to be exposed. It also usually contains information on suitable types of PPE (personal protective equipment)

i. Section 9 is self-explanatory. It describes the physical and chemical properties, such as the appearance of the chemical, the product odor and other characteristics as listed on the MSDS.

j. Section 10 is also largely self-explanatory. The section describes the product stability and reactivity, the thermal decomposition/conditions to be avoided, materials to be avoided, oxidizing agents, and known dangerous reactions.

k. Section 11 outlines the risks to which you may be exposed when using the chemical. It is, therefore, a section of crucial importance!

l. Section 12 describes indicator species that were used in ecological toxicity testing.

m. Section 13, which deals with disposal, is often not sufficiently detailed for you to be able to undertake disposal yourself. If you need to dispose of the chemical after use, ensure that you know how to do this safely.

n. Section 14 gives transport information, generally as a list of codes indicating the dangers associated with the chemical (flammable, radioactive, significant toxicity, etc.) and the type of transport which may be used. There are usually UN hazard codes given in this section.

o. Section 15 lists the hazard codes which indicate the principle hazards associated with the chemical and the precautions which should be taken.

p. Finally, section 16 provides any additional information, such as the name of the person preparing the data sheet, a list of references from which data have been drawn, disclaimers, and so forth.

**Section 1 - Product and Company Identification**
**HYDROCHLORIC ACID (MURIATIC ACID)**

Product Identification: HYDROCHLORIC ACID (MURIATIC ACID)
Date of MSDS: 01/20/1986 Technical Review Date: 07/11/1988
FSC: 6810 NIIN: LIIN: 00B190008
Submitter: B DT
Status Code: C
MFN: 01
Article: N

**Manufacturer's Information**

Manufacturer's Name: SUNNYSIDE CORPORATION
Post Office Box: N/K
Manufacturer's Address1: 225 CARPENTER AVE.
Manufacturer's Address2: WHEELING, IL 60090
Manufacturer's Country: US
General Information Telephone: 312541-5700
Emergency Telephone: 800424-9300
Emergency Telephone: 800424-9300
MSDS Preparer's Name: N/K
Proprietary: N
Reviewed: Y
Published: Y
CAGE: 9J570
Special Project Code: N

**Contractor Information**

**Contractor's Name:** BERKMANN MFG CO
**Contractor's Address1:** N/P
**Contractor's Address2:** CHICAGO, IL 60600
**Contractor's Telephone:** N/P
**Contractor's CAGE:** 95570

**Contractor Information**

**Contractor's Name:** SUNNYSIDE CORP
**Contractor's Address1:** 225 CARPENTER AVE
**Contractor's Address2:** WHEELING, IL 60090-6009
**Contractor's Telephone:** 847-541-5700
**Contractor's CAGE:** 9J570

Figure 1-3. Material Safety Data Sheet (continued).

**Section 2 - Composition/Information on Ingredients**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Ingredient Name:** HYDROGEN CHLORIDE (HYDROCHLORIC ACID) (SARA III)
**Ingredient CAS Number:** 7647-01-0 **Ingredient CAS Code:** M
**RTECS Number:** MW4025000 **RTECS Code:** M
**=WT: =WT Code:**
**=Volume: =Volume Code:**
**>WT: >WT Code:**
**>Volume: >Volume Code:**
**<WT: <WT Code:**
**<Volume: <Volume Code:**
**% Low WT: % Low WT Code:**
**% High WT: % High WT Code:**
**% Low Volume: % Low Volume Code:**
**% High Volume: % High Volume Code:**
**% Text:** 27.9%
**% Environmental Weight:**
**Other REC Limits:** N/K
**OSHA PEL:** C 5 PPM **OSHA PEL Code:** M
**OSHA STEL: OSHA STEL Code:**
**ACGIH TLV:** C 5 PPM; 9192 **ACGIH TLV Code:** M
**ACGIH STEL:** N/P **ACGIH STEL Code:**
**EPA Reporting Quantity:** 5000 LBS
**DOT Reporting Quantity:** 5000 LBS
**Ozone Depleting Chemical:** N

**Section 3 - Hazards Identification, Including Emergency Overview**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Health Hazards Acute & Chronic:** NONE EXPECTED WHEN GOOD HYGIENIC PRACTICES ARE EMPLOYED.

**Signs & Symptoms of Overexposure:**
HYDROCHLORIC ACID IS CAPABLE OF IRRITATING AND BURNING THE SKIN AND MUCOUS MEMBRANES, THE SEVERITY DETERMINED BY THE CONCENTRATION OF THE SOLUTION AND DURATION OF EXPOSURE. CONTACT W/EYES MAY CAUSE SEVERE BURNS, VISUAL IMPAIRMENT OR LOSS OF SIGHT MAY RESULT. INGESTION CAUSES SEVERE BURNS OF MOUTH, ESOPHAGUS AND STOMACH.

**Medical Conditions Aggravated by Exposure:**
N/K

Figure 1-3. Material Safety Data Sheet (continued).

**LD50 LC50 Mixture:** N/K
**Route of Entry Indicators:**
**Inhalation:** YES
**Skin:** YES
**Ingestion:** YES
**Carcinogenicity Indicators**
**NTP:** N/P
**IARC:** N/P
**OSHA:** N/P
**Carcinogenicity Explanation:** N/K

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**Section 4 - First Aid Measures**
**HYDROCHLORIC ACID (MURIATIC ACID)**

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**First Aid:**
EYE CONTACT: IMMEDIATELY FLUSH EYES W/A DIRECTED STREAM OF WATER FOR 15 MIN, HOLDING EYELIDS APART TO ENSURE COMPLETE IRRIGATION OF ALL EYE AND LID TISSUE. CONTACT LENSES SHOULD NOT BE WORN. SKIN CONTACT: FLUSH CONTAMINATED SKIN W/SOAP AND WATER. USE SAFETY SHOWER IF LARGE AREAS OF THE BODY ARE CONTAMINATED. INGESTION: GIVE LARGE QUANTITY OF WATER. DO NOT INDUCE VOMITING. IF INHALED: REMOVE TO FRESH AIR.

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**Section 5 - Fire Fighting Measures**
**HYDROCHLORIC ACID (MURIATIC ACID)**

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**Fire Fighting Procedures:**
USE SELF-CONTAINED BREATHING APPARATUS AND FULL PROTECTIVE EQUIPMENT.
**Unusual Fire or Explosion Hazard:**
REACTS WITH ACTIVE METALS (POTASSIUM, SODIUM, CALCIUM, POWDERED ALUMINUM, ZINC and MAGNESIUM) TO PRODUCE FLAMMABLE HYDROGEN.
**Extinguishing Media:**
USE WATER SPRAY, FOG, FOAM, DRY CHEMICALS, CARBON DIOXIDE OR OTHER AGENTS AS APPROPRIATE FOR SURROUNDING FIRE.
**Flash Point: Flash Point Text:** N/R
**Auto-ignition Temperature:**
**Auto-ignition Temperature Text:** N/A
**Lower Limit(s):** N/R
**Upper Limit(s):** N/R

Figure 1-3. Material Safety Data Sheet (continued).

**Section 6 - Accidental Release Measures**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Spill Release Procedures:**
CONTAIN SPILL AND PUMP INTO MARKED CONTAINERS FOR RECLAMATION OR DISPOSAL. CLEAN UP SPILL AREA UNTIL DRY AND THEN FLUSH THOROUGHLY WITH WATER.

**Section 7 - Handling and Storage**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Handling and Storage Precautions:**

**Other Precautions:**

**Section 8 - Exposure Controls & Personal Protection**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Respiratory Protection:**
USE NIOSH/MSHA APPROVED ORGANIC VAPOR ACID-GAS RESPIRATOR FOR AREAS WHERE AIRBORNE EXPOSURE IS EXCESSIVE.
**Ventilation:**
GENERAL ROOM VENTILATION TO KEEP CONCENTRATION BELOW APPLICABLE OSHA SAFETY &HEALTH REQUIREMENTS. LOCAL FOR VAPOR EMISSION
**Protective Gloves:**
RUBBER OR NEOPRENE GLOVES
**Eye Protection:** CHEMICAL SAFETY GOGGLES
**Other Protective Equipment:** EYE WASH FACILITY SHOULD BE IN CLOSE PROXIMITY. RUBBER COVERALLS, SHOES, AND EMERGENCY SHOWER AVAILABILITY.
**Work Hygienic Practices:** WASH THOROUGHLY AFTER CONTACT. WASH PROTECTIVE CLOTHING PRIOR TO RE-USE.
**Supplemental Health & Safety Information:** VAPORS HAVE AN IRRITATING EFFECT ON THE RESPIRATORY TRACT. AVOID BREATHING VAPORS. DO NOT GET IN EYES OR ON SKIN OR CLOTHING. KEEP CONTAINERS CLOSED. PROTECT CONTAINERS FROM PHYSICAL DAMAGE. STORE IN COOL, WELL VENTILATED PLACE, SEPARATED FROM ALL OXIDIZING MATERIALS. KEEP LIGHTS, FIRE AND SPARKS AWAY FROM CONTAINER OPENINGS.

Figure 1-3. Material Safety Data Sheet (continued).

**Section 9 - Physical & Chemical Properties**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**HCC:**
**NRC/State License Number:**
**Net Property Weight for Ammo:**
**Boiling Point: Boiling Point Text:** 178 DG.
**Melting/Freezing Point: Melting/Freezing Text:** N/K
**Decomposition Point: Decomposition Text:** N/K
**Vapor Pressure:** N/K **Vapor Density:** >THAN AIR
**Percent Volatile Organic Content:**
**Specific Gravity:** N/K
**Volatile Organic Content Pounds per Gallon:**
**pH:** 1.0
**Volatile Organic Content Grams per Liter:**
**Viscosity:** N/P
**Evaporation Weight and Reference:** SLOWER THAN ETHER
**Solubility in Water:** INFINITE
**Appearance and Odor:** N/K
**Percent Volatiles by Volume:** 100%
**Corrosion Rate:** N/K

**Section 10 - Stability & Reactivity Data**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Stability Indicator:** YES
**Materials to Avoid:**
AVOID BASE AND CORROSIVE MATERIALS.
**Stability Condition to Avoid:**
AVOID CONTACT W/METALS AND STRONG OXIDIZERS.
**Hazardous Decomposition Products:**
FLAMMABLE HYDROGEN GAS CAN BE PRODUCED BY THE REACTION W/MOST METALS. CHLORINE GAS IS RELEASED BY MIXING W/STRONG OXIDIZE
**Hazardous Polymerization Indicator:** NO
**Conditions to Avoid Polymerization:**
N/K

Figure 1-3. Material Safety Data Sheet (continued).

**Section 11 - Toxicological Information**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Toxicological Information:**
N/P

**Section 12 - Ecological Information**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Ecological Information:**
N/P

**Section 13 - Disposal Considerations**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Waste Disposal Methods:**
DISPOSE OF SPILLED OR WASTE PRODUCT CONTAMINATED SOIL AND OTHER CONTAMINATED MATERIALS IN LICENSED LANDFILL OR TREATMENT FACILITY IN ACCORDANCE WITH ALL LOCAL, STATE AND FEDERAL REGULATIONS.

**Section 14 - MSDS Transport Information**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Transport Information:**
N/P

**Section 15 - Regulatory Information**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**SARA Title III Information:**
N/P
**Federal Regulatory Information:**
N/P
**State Regulatory Information:**
N/P

Figure 1-3. Material Safety Data Sheet (continued).

**Section 16 - Other Information**
**HYDROCHLORIC ACID (MURIATIC ACID)**

**Other Information:**
N/P

**HAZCOM Label Information**

**Product Identification:** HYDROCHLORIC ACID (MURIATIC ACID)
**CAGE:** 95570
**Assigned Individual:** N
**Company Name:** BERKMANN MFG CO
**Company PO Box:**
**Company Street Address1:** N/P
**Company Street Address2:** CHICAGO, IL 60600 NK **Health Emergency**
**Telephone:** 800424-9300
**Label Required Indicator:** Y
**Date Label Reviewed:** 12/16/1998
**Status Code:** C
**Manufacturer's Label Number:**
**Date of Label:** 12/16/1998
**Year Procured:** N/K
**Organization Code:** G
**Chronic Hazard Indicator:** N/P
**Eye Protection Indicator:** N/P
**Skin Protection Indicator:** N/P
**Respiratory Protection Indicator:** N/P
**Signal Word:** N/P
**Health Hazard:**
**Contact Hazard:**
**Fire Hazard:**
**Reactivity Hazard:**

Figure 1-3. Material Safety Data Sheet (concluded).

## 1-4. WORK AREA

To prevent accidents, the work areas must be kept clean and free of clutter at all times. There is to be no storage of equipment in the aisles of the work area or where it blocks emergency exits. Laboratory chairs must be removed from the aisles when not being used. No smoking, eating, drinking, wearing contacts, or applying of makeup is allowed in the work area and there should be a designated area for breaks. Hands should always be washed prior to eating, smoking, or drinking, regardless of the laboratory procedure or use of plastic gloves. All wastes must be placed in appropriately labeled containers (e.g., paper and plastic, glass and metal, chemical, radioactive, or biological).

## Section II. VOLATILE AND HAZARDOUS MATERIALS

### 1-5. VOLATILE FLAMMABLES

Misuse of volatiles (e.g., alcohol, acetone, or ether) significantly increases the likelihood of a fire in the laboratory. Volatiles are substances that can form vapors at relatively low temperatures. Knowledge and practice of the safety rules, which directly pertain to the wise use of volatile flammables, will prevent most accidents.

a. **Storage.** Volatile flammables are stored in safety storage cabinets that are vented or in explosion proof refrigerators. Each is properly labeled as to its contents and whether it is explosion proof. Flammable volatiles are never stored on shelves above work benches, near electrical equipment, or with oxidizers, such as hydrogen peroxide. No more than sixty gallons of volatiles may be stored in each cabinet or refrigerator. A maximum of five gallons of volatile substances can be stored outside each cabinet per room and they must be stored in Department of Transportation (DOT) approved containers.

b. **Handling.** "No Smoking" signs must be present in all areas where flammables are being used. There must be adequate ventilation and no open flame in the area. Use of a fume hood is recommended when handling flammables. If a solution containing flammable material is being evaporated, it should be performed on a steam bath or sand-filled mantel to control evaporation temperature and superheating, which could result in explosion. Special precautions must be taken when using ethyl ether, which can be ignited by many heat sources, including a hot plate.

c. **Disposal.** Disposal of flammable solvents in the sink or sewers, generally, is not allowed. Small amounts, which are miscible with water, may be disposed of followed by large quantities of water. All other flammables should be stored in approved safety cans until they can be properly disposed.

d. **In Case of Fire.** The phone number of the fire department must be posted next to all phones within the laboratory. It is mandatory to have a fire evacuation plan posted in several areas showing all routes of evacuation. All evacuation routes must be free of physical obstruction, such as refrigerators and storage cabinets. Every technician must know the location of the fire alarm station and be instructed concerning its use. Each laboratory must have the necessary equipment to put out or confine a fire. Various types of fire extinguishers are available and each technician must know their mode of operation and type of fire for which each is used. Dry chemical fire extinguishers are the best all purpose extinguishers. Fire blankets must be available and all personnel familiar with their use.

## 1-6. HAZARDOUS MATERIALS

a. **Chemicals.**

**CAUTION:** All chemicals in the laboratory must be considered to be poisonous.

(1) Storage. Certain chemicals should never be stored together. Acids are not stored with bases, oxidizing agents are not stored with reducing agents, and acids are not stored with volatiles. Once a chemical solution is prepared, it is typically stored in an unbreakable plastic bottle and labeled as to its date of preparation, contents, and the initials of the technician who prepared it. Do not taste or inhale vapors of unknown chemicals.

(2) Spillage. When a chemical is spilled, it must be cleaned up immediately. If acids are spilled, the area should be cleaned with sodium bicarbonate and thoroughly flushed with water. If bases are spilled, clean with a 1:5 dilution of acetic acid and flush with water. When cleaning these spills, rubber gloves and protective clothing are recommended. If you spill strong acids or bases on yourself or your clothing, immediately flush with water utilizing the emergency shower or eyewash, if necessary.

(3) Preparation of solutions. When preparing solutions of strong acids or bases, technicians should wear a plastic apron as well as safety goggles or a face shield. Always add acid slowly to water but never water to acid which will limit heat production. If toxic fumes are produced during the preparation of a reagent, this reagent must be prepared under a fume hood. Adequate ventilation is a must for all preparation rooms to prevent inhalation of toxic fumes.

(4) Use of solutions. To prevent swallowing of poisonous or caustic reagents, pipeting by mouth is never allowed. There are accurate, mechanical pipeting devices available for this use. If, for some reason, a chemical is swallowed, refer to the MSDS and contact a physician immediately.

b. **Mercury**. Mercury is still used in some thermometers and in some reagents as mercuric salt. Many people forget that mercury is a health hazard if it is not properly handled and disposed. Small amounts of spilled mercury in a poorly ventilated area can have cumulative toxic effects. Large quantities of mercury containing reagents (e.g., Trinder's Salicylate reagent) poured down sinks can contaminate water supplies. Accidental spillage from broken thermometers should be cleaned carefully with sulfur until no droplets remain. Commercial kits are available for cleanup. Used reagent is collected in plastic containers, made slightly acid with acetic acid, and thioacetamide added (10 g/L). Over a period of time, the mercury will precipitate as mercuric sulfide, which can be disposed by burial in accordance with local regulations. The remaining supernatant can be safely poured down the drain.

c. **Azide**. Sodium azide is a preservative used in a variety of commercially produced reagents. When poured down the sink, azide can form explosive salts as it may react with the metal of the drainage pipes. These salts can be detonated by mechanical shock. When disposing of solutions containing azide down the sink, it is recommended to rinse with copious amounts of water.

d. **Gas Cylinders**. If gas cylinders are used in the clinical laboratory, they must be secured to the laboratory bench or to the wall so they will not overturn. During storage, the cylinder cap must always be on the tank to prevent accidentally breaking the outlet valve of a full cylinder. It is recommended that all gas lines be color coded (same as the tank) and labeled to prevent accidentally hooking up the wrong lines. No smoking signs are to be posted in the area where flammable gases are used.

**CAUTION**: To prevent an explosion, cylinder gauges, especially oxygen gauges, should NEVER be oiled.

e. **Radioactive Materials**. On occasion radioimmunoassay techniques may be used in the laboratory, technicians may be required to handle radioactive materials. Although the amount of radioactive material is small and the half-life is generally short, proper safety procedures must be adhered to and protective clothing worn. Technicians must be familiar with the type of radiation, half-life of the material used, and any shielding required. Under no circumstances is eating or drinking allowed in areas containing radioactive materials. All waste materials must be disposed according to federal regulations. Spilled material must be cleaned immediately and the area thoroughly decontaminated. Consult your local SOP and safety office for additional guidance.

**CAUTION**: Under no circumstances is pipeting by mouth allowed in this area. Hand signs must be posted "warning of radioactive area."

f. **Specimens**. ALL BIOLOGICAL FLUIDS SUCH AS CEREBROSPINAL FLUID (CSF), BLOOD, URINE, AND SPUTUM SHOULD BE CONSIDERED TO CONTAIN PATHOGENIC ORGANISMS AND BE TREATED AS INFECTIOUS MATERIAL. To prevent the release of aerosols, test tubes should be capped and centrifuges not opened until they come to a complete stop.

**WARNINGS**

NEVER PIPETTE BY MOUTH TO AVOID EXPOSURE TO HIV, HEPATITIS B, OR OTHER INFECTIOUS MATERIALS AND CORROSIVE OR TOXIC AGENTS.

ALWAYS WEAR PROTECTIVE GLOVES, APRONS, AND EYE PROTECTION.

WASH HANDS FREQUENTLY.

g. **Glassware**. To prevent cuts, all cracked, chipped, or broken glassware must be discarded in an appropriately labeled container. All beakers and flasks used with corrosive or toxic substances are rinsed with water prior to being placed with soiled glassware to be washed. One of the most common injuries is received by improperly trying to insert a pipet or glass rod into a Propipet® or rubber stopper. To prevent lacerations or puncture wounds, wet the glass end with deionized water or glycerin, hold the glassware near the end to be inserted, and **GENTLY**, with a twisting action, insert the glassware into the Propipet® or rubber stopper. A set of heat resistant gloves should be available for use by all technicians for handling hot glassware. Large glass bottles or flasks are always supported by the base when carried. A first aid kit must be present in all laboratories for use in case of minor injuries.

h. **Electrical**. Electrical hazards are present in all chemistry laboratories. To avoid electrical shock, all equipment must be grounded and should be checked to ensure proper grounding on a periodic basis by medical maintenance personnel. Any frayed or worn cords must be replaced immediately. Extension cords are prohibited; however, power strips may be used if more than one outlet is required. Mercury switches must be installed in all areas where volatile flammables are used or stored. There should be sufficient lighting in all working areas to ensure at least 60 foot-candles at bench top level. If the technician is adjusting the instrument (e.g., changing an exciter lamp), the instrument must be unplugged. Do not plug in equipment if your hands are wet.

**Continue with Exercises**

## EXERCISES, LESSON 1

**INSTRUCTIONS**: Answer the following exercises by marking the lettered response that best answers the question, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Medical laboratory specialists are now exposed to potential clinical health and:

   a. Precautions.

   b. Different standards.

   c. Safety hazards.

   d. None of the above.

   e. a and b above.

2. Which of the following statements is the most appropriate for laboratory safety?

   a. Acids and bases should be stored together because in an emergency one could be added to the other to neutralize both.

   b. Alcohol should be stored in 100 gallon containers to facilitate rapid removal in the event of fire.

   c. Smoking should be allowed only in chemical storage areas.

   d. Work areas should be clean and free of clutter.

3. What is each clinical laboratory required to have?

    a. A formal safety program.

    b. A safety SOP.

    c. An appointed safety officer and/or NCO to administer the safety program.

    d. All of the above.

4. A technician, new to the laboratory, can easily identify potential hazard areas by the proper location of:

    a. Lab safety notes.

    b. Hazard warning signs.

    c. Showers and fire extinguishers.

    d. Notes and messages on the bench tops.

5. What information should the SOP, one of the most important items in the laboratory, contain?

    a. Safety practices and precautions.

    b. Laboratory layout and emergencies.

    c. The use and operation of equipment and supplies.

    d. The laboratories standard policies and procedures.

    e. All of the above.

6. As part of the continuing safety education program, what type of hands-on training is needed to assist medical laboratory specialists in remaining current with safety procedures?

   a. Reading.

   b. Updating documentation.

   c. Actual practice drills.

   d. Attending conferences.

7. Which standard regulations should be followed when disposing of hazardous or infectious material?

   a. Your standards.

   b. Local regulations.

   c. OSHA regulations.

   d. b and c above.

8. Which items are to be checked when inspecting chemicals?

   a. Labeling of containers housing chemicals.

   b. Types of cabinets storing chemicals.

   c. a and b.

   d. Brightness in color of all chemicals.

9. For a successful safety program, how often should safety inspections be conducted in the laboratory?

   a. Daily.

   b. Weekly and/or monthly.

   c. Quarterly.

   d. Annually.

10. Which NFPA warning sign identifies the degree of flammability on a 0-4 scale, 4 being extremely flammable (will ignite at temperatures below 73°F).

    a. Red.

    b. Blue.

    c. Pink.

    d. White.

11. What type of NFPA hazard does the yellow warning sign identify?

    a. The health hazards, 0-6 scale, reserved for the most hazardous material at 6.

    b. Ignition of extremely flammable material (will ignite at temperatures above 73°F).

    c. The reactivity or instability of the hazardous material.

    d. Special hazards for NCOs in the laboratory.

12. In the NFPA Hazardous Material Identification System, what type of hazard does a sign with a blue diamond positioned to the left identify?

    a. Flammable.

    b. Health.

    c. Reactivity.

    d. Contact.

    e. None of the above.

13. What is the purpose for using MSDS?

    a. For manufactures to identify the physical and health dangers of using and handling their products.

    b. For manufactures to identify the hazardous material, its toxicity, and the appropriate handling instructions.

    c. To list all chemicals, hazardous or not, used within the laboratory.

    d. a and b.

14. If a respirator is needed, in which section of the MSDS will this information be located?

    a. Handling and Storage.

    b. Exposure Controls & Protective Equipment.

    c. First Aid Measures.

    d. Disposal Considerations.

15. Which section of the MSDS contains the hazardous decomposition products that can be produced by the chemical?

    a. Physical & Chemical Properties.

    b. Ecological Information

    c. Stability and Reactive data.

    d. Toxicological Information.

16. If the chemical has come in contact with your eyes, what section of the MSDS would you consult?

    a. Toxicology Information.

    b. Physical and Chemical Properties.

    c. First Aid Measures.

    d. Composition/Information on Ingredients

17. Solvents are considered to be volatile flammables if they:

    a. Form vapors at relatively low temperatures.

    b. Ignite at temperatures above $100^{o}$ F.

    c. Ignite at temperatures below $100^{o}$ F.

    d. a and b.

    e. a and c.

18. A technician is concerned about the long term effects of using xylene in a room that has adequate ventilation. Which section of the MSDS will tell the maximum amount of airborne xylene one should be exposed to during the workday?

    a. Component and contaminants.

    b. Health hazard data.

    c. Special precautions.

    d. Physical data.

19. Which statement is NOT a consideration in a formal laboratory safety program?

    a. All new personnel to the laboratory are required to read the lab safety SOP.

    b. Safety topics should be included in the continuing education program to remind personnel of potential hazards.

    c. Check safety equipment after it has been used by untrained personnel.

    d. Material Safety Data Sheets for all chemicals and reagents must be maintained and available for all personnel.

20. When disposing of waste in the laboratory work area, where must waste be placed?

    a. In the aisles.

    b. Inside garbage cans with lids.

    c. In appropriately labeled containers.

    d. In open trash cans or waste baskets.

21. Which statement is correct concerning the storage of volatile flammables?

    a. Store volatile flammables on shelves below work benches.

    b. Store volatile flammables near electrical equipment.

    c. Store volatile flammables with oxidizers such as hydrogen peroxide.

    d. Store volatile flammables to the maximum of no more than sixty gallons of volatiles per cabinet or refrigerator.

22. Which of the following statements concerning the storage of volatile flammables is true?

    a. No more than 5 gallons per room may be stored outside of safety cabinets in approved containers.

    b. Small quantities may be kept on the shelves above the workbench for convenience.

    c. Hydrogen peroxide may be stored with volatile flammables if there is no other room for storage.

    d. Safety cabinets do not need to be vented if the door is opened frequently.

23. Flammable solvents should be disposed of:

    a. In small, miscible amounts, with water followed by large amounts of water through the sink or sewer.

    b. In large amounts, with water followed by large amounts of water through the sink or sewer.

    c. Regularly through the sink or sewer.

    d. By can, approved type or not.

24. If fire occurs in a laboratory, which statement describes the types of extinguishers that should be used to confine or put it out?

    a. A half full paint can or a blanket.

    b. A blanket, the type of extinguisher for that particular type of fire, or a dry chemical all purpose fire extinguisher.

    c. A blanket or volatile substance.

    d. None of the above.

25. Chemicals are hazardous materials. Certain chemicals should NEVER be stored:

    a. Together.

    b. In adequate ventilation.

    c. In explosive proof containers.

    d. As per the SOP.

26. For safety reasons, what procedures should be followed when storing hazardous chemicals?

    a. Store acids with bases.

    b. Store acids separately from bases.

    c. Store oxidizing agents away from reducing agents.

    d. b and c above.

    e. a, b, and c above.

27. Once a chemical solution is prepared, in what should it be stored?

    a. A breakable container labeled as to its date of preparation, contents, and initialed by the technician who prepared the solution.

    b. An unbreakable plastic bottle, labeled as to its date of preparation, contents, and initialed by the technician who prepared the solution.

    c. A porous, breakable container that will allow vapors to be inhaled.

    d. A volatile container with volatile materials labeled as to its date of preparation, contents, and initialed by the technician who prepared the solution.

28. When preparing a chemical solution and adding an acid, you should add the acid:

    a. Quickly.

    b. Forcefully.

    c. Slowly.

    d. Sporadically.

29. What should you do if a chemical acid spill occurs?

    a. Clean the area immediately with acetic acid.

    b. Clean the area immediately with soap and water.

    c. Let the stain sit until it dries and then use sodium bicarbonate.

    d. a or c.

    e. Clean the area immediately with sodium bicarbonate and thoroughly flush with water.

30. What should you do if you spill a weak base?

    a. Clean the area immediately with pure acetic acid.

    b. Clean immediately with a 1:5 dilution of acetic acid and flush it with water.

    c. Clean the area immediately with soap and water.

    d. Let the stain sit until it dries, and then use sodium bicarbonate.

31. All biological fluids such as cerebrospinal fluid, blood, urine and sputum are potential hazards. Consider them to contain:

    a. Organic substances.

    b. Bacterial organisms.

    c. Pathogenic organisms.

    d. Gases.

32. Which of the following statements about mercury is true?

    a. Reagents, containing mercury or mercuric salts, may be safely poured down the drain if flushed with copious amounts of water.

    b. Large amounts of mercury may have cumulative toxic effects in poorly ventilated areas.

    c. Droplets of mercury in cracks of the floor or the bench top pose no significant safety hazard.

    d. After treating mercuric reagents with thioacetamide, the resulting precipitate may be dumped with non-hazardous waste.

33. If a corrosive base is accidentally spilled on the floor, which of the following actions should be taken?

    a. Neutralize the base with sodium bicarbonate and thoroughly flush with water.

    b. Put on necessary protective clothing like gloves and an apron.

    c. Clean the spill with 1:5 dilution of acetic acid and thoroughly flush with water.

    d. Use absorbent towels to clean up the base and flush with water

    e. b and c above.

34. Which of the following statements best describes mercury in thermometers?

    a. It is used in some reagents as mercuric salt.

    b. If not properly handled and disposed, small spills in a poorly ventilated area can have cumulative toxic effects.

    c. Large quantities of mercury containing reagents (e.g., Trinder's Salicylate reagent) if poured down a sink can contaminate the water supply.

    d. Clean the spillage from broken thermometers carefully with sulfur until no droplets remain.

    e. a and b above.

    f. a, b, c, and d above.

35. Which chemical, when poured down a sink, will form an explosive salt and can detonate?

    a. Mercury.

    b. Reagent.

    c. Azide.

    d. Sodium chloride.

36. Whenever gas cylinders are present in a clinical laboratory, what is/are the standard procedure(s)?

    a. Secure them to the floor.

    b. Color code the gas lines the same color as the tank to prevent accidentally hooking up the wrong lines.

    c. Post no smoking signs and refrain from smoking in the laboratory because the flammable gases will ignite.

    d. a and b above.

    e. b and c above.

37. When using or storing gas cylinders, what must NEVER be done?

    a. Secure them to the wall or bench.

    b. Place the cylinder cap on the tank.

    c. Oil the cylinder or oxygen gauges.

    d. Follow and use safety procedures always.

38. When working with radioactive materials, you must be familiar with the:

    a. Type of radiation, life of the material used, and any shielding required.

    b. Type of clothing worn, half-life of the material used, and any shielding required.

    c. Type of radiation, half-life of the material used, and any shielding required.

    d. Short life, type of radiation, and any shielding required.

39. When working with specimens, one of the things that a technician must NEVER do is to:

    a. Wear protective gloves.

    b. Work with spinal fluid.

    c. Pipet by mouth.

    d. Treat each specimen seriously.

40. To prevent infection, which safety procedures should be followed when working with specimens?

    a. Wear protective gloves and eye glasses and wash hands frequently before eating or smoking. Only open centrifuges after they come to a complete stop to prevent the release of aerosols.

    b. Wear protective gloves and eye protection and wash hands frequently after eating or smoking. Only open centrifuges after they come to a complete stop to prevent the release of aerosols.

    c. Wear protective gloves and eye protection, and wash hands frequently before eating or smoking. Only open centrifuges as their spin slows down to prevent the release of aerosols.

    d. Wear protective gloves and eye protection, and wash hands frequently before eating or smoking. Only open centrifuges after they come to a complete stop to prevent the release of aerosols.

41. Which statements are correct concerning electrical hazards?

    a. Electrical equipment must be grounded and checked periodically.

    b. Frayed or worn cords must be taped immediately.

    c. Extension cords are prohibited; however, power strips may be used if more than one outlet is required.

    d. Mercury switches must be installed in all areas where volatile flammables are used or stored.

    e. When adjusting or changing an exciter lamp, it must be unplugged. NEVER plug in equipment if your hands are wet.

    f. a, c, d, and e above.

42. What should you do to prevent a common laceration or puncture wound when using the Propipet® or rubber stopper?

    a. Wet the glass end with deionized water or glycerin, hold the glassware near the end to be inserted and **GENTLY**, with a twisting action, insert the glassware into the Propipet® or rubber stopper.

    b. Use a set of heat resistant gloves.

    c. Use with a dry glass instead of wet one.

    d. Keep a first aid kit handy for those minor injuries.

**Check Your Answers on Next Page**

## SOLUTIONS TO EXERCISES, LESSON 1

1. c (para 1-1)
2. d (para 1-4)
3. d (para 1-2)
4. b (para 1-2c)
5. e (para 1-2a)
6. c (para 1-2c(1))
7. d (para 1-2b)
8. c (para 1-2b)
9. b (para 1-2b)
10. a (para 1-2c(2))
11. c (para 1-2c(3))
12. b (para 1-2c(1), figure 1-2)
13. d (para 1-3)
14. b (para 1-3i, figure 1-3)
15. c (para 1-3a, figure 1-3)
16. c (para 1-3e, figure 1-3)
17. e (paras 1-3d, 1-5)
18. a (para 1-3b)
19. c (para 1-3h)
20. c (para 1-4)
21. d (para 1-5a)
22. a (para 1-5a)

23. a (para 1-5c)

24. b (pare 1-5d)

25. a (para 1-6a(1))

26. d (para 1-6a(1))

27. b (para 1-6a(1))

28. c (para 1-6a(3))

29. e (para 1-6a(2))

30. b (para 1-6a(2))

31. c (para 1-6f)

32. b (para 1-6b)

33. c (para 1-6a(2))

34. f (para 1-6b)

35. c (para 1-6c)

36. e (para 1-6d)

37. c (para 1-6d CAUTION)

38. c (para 1-6e)

39. c (para 1-6f, WARNING)

40. a (para 1-6f, WARNING)

41. f (para 1-6h)

42. a (para 1-6g)

**End of Lesson 1**

## LESSON ASSIGNMENT

| | |
|---|---|
| **LESSON 2** | Collection, Preservation, and Shipment of Specimens. |
| **TEXT ASSIGNMENT** | Paragraphs 2-1 through 2-14. |
| **LESSON OBJECTIVES** | After completing this lesson, you should be able to: |

2-1. Select the statement which best describes the collection and preservation of blood specimens and types of specimen collections.

2-2. Select the statement which best describes the collection and preservation method of choice for blood (serum/plasma), urine, fecal, cerebrospinal fluid, serous, synovial fluids, and amniotic fluid specimens.

2-3. Define correctly a chelator.

2-4. Select the statement which best describes the anticoagulants used in the collection of blood specimens for specific types of tests.

2-5. Select the statement which correctly states the tube color used for specific anticoagulants.

2-6. Select the statement which best describes the difference between serum and plasma.

2-7. Select the statement which best describes the proper collection of a 24-hour urine specimen and common sources of error.

2-8. Select the statement which best describes the collection variables that will result in erroneous or variable test results.

2-9. Select the statement which best describes the criteria for unacceptable samples.

2-10. State the proper specimen chain of custody.

2-11. Select the statement which best describes the use of DD Form 1323.

**SUGGESTION** After studying the assignment, complete the exercises at the end of this lesson.

# LESSON 2

# COLLECTION, PRESERVATION, AND SHIPMENT OF SPECIMENS

## Section I. COLLECTION AND PRESERVATION OF SPECIMENS

### 2-1. INTRODUCTION

The credibility and reputation of the laboratory lies in its ability to perform accurate and precise analyses of specimens. A large part of maintaining this credibility is the laboratory's ability to maintain the chemical integrity of the specimen from the patient to the time the results are posted on the report sheet. To ensure the chemical integrity of a specimen (blood, urine, etc.), a current set of instructions must be readily available to all technicians (as well as ward personnel) which dictates the type of specimen, type of anticoagulant used (if any), and the amount of specimen required for the test(s) being requested. This set of instructions also must include any special instructions that are required in the drawing of the specimen; for example, notification of the laboratory 30 minutes prior to drawing the sample.

### 2-2. COLLECTION AND PRESERVATION OF BLOOD SPECIMENS

When a test is requested, the clinician is interested in the laboratory results for one reason, that is, how do the results reflect the condition of the patient. However, one of the most common errors in the clinical laboratory is the drawing of the incorrect type of specimen.

a. **Types of Specimens.**

(1) Random specimen. This type of specimen may be collected any time of the day or night to assess a patient's condition at the time of collection. Most random specimens have little diagnostic value except to establish a baseline to follow the course of treatment.

(2) Fasting specimen. The fasting specimen is by far the most common type of specimen requested. This specimen is drawn after the patient has avoided the intake of food (fasted) for at least eight hours prior to the drawing of the specimen. When non-fasting specimens are drawn, the technician must be aware that the following tests will be affected: (1) glucose and triglyceride concentrations will be increased, (2) phosphate concentrations will be decreased, and (3) turbidity from increased chylomicrons may interfere with colorimetric spectrophotometric procedures.

(3) Two-hour postprandial (pp) specimen. The two-hour postprandial (2 hr pp) is drawn exactly two hours after a patient has completed a meal or been given a high carbohydrate drink (Glucola). This type of specimen is commonly requested in conjunction with glucose tolerance testing.

b. **Types of Anticoagulants.** There are several types of anticoagulants used in the clinical chemistry laboratory. It is of utmost importance to use the proper anticoagulant (if needed) to prevent chemical interference of specific test procedures and to prevent degradation of certain analytes if testing cannot begin immediately. In collecting blood, the most commonly available method involves the use of Vacutainer tubes, which are color coded to indicate the type of anticoagulant present. If these tubes are not available, the technician must fill the collection syringe with the proper anticoagulant or transfer the whole blood to a sterile, chemically clean test tube that contains the desired anticoagulant. Regardless of the method used to collect the blood, the technician must mix the blood specimen with the anticoagulant immediately by gentle inversion. The tube is then centrifuged at approximately 2000 revolutions per minute (rpm) for 10 minutes and the supernatant fluid (plasma) removed as soon as possible.

(1) Heparin--green top Vacutainer. Heparin is an anticoagulant that is normally present in blood but in concentrations less than required to prevent the coagulation of freshly drawn whole blood. Heparin acts as an anticoagulant by preventing the conversion of prothrombin to thrombin. It is the most widely used anticoagulant and causes the least interference. Heparin is commercially available in sodium, potassium, lithium, and ammonium salts and such preparations have been shown to contain phosphate contamination. Heparinized whole blood cannot be used for phosphate determinations or the test that corresponds to the heparin salt employed.

(2) Ethylenediamine tetraacetic acid (EDTA)--lavender top Vacutainer. EDTA is an example of a chelating anticoagulant. Chelators are substances that bind metal ions. EDTA binds with calcium ions which are essential for the coagulation process. This anticoagulant is available as disodium and dipotassium salts, the latter being preferred because of its greater solubility in aqueous solutions. It has been reported that EDTA does not interfere with glucose, urea, or creatinine determinations; however, it will increase prothrombin time. EDTA is most commonly used in hematology since it preserves the cellular components of the blood. EDTA cannot be used when calcium determinations are required or the test that corresponds to the salt being used.

(3) Oxalates-- black top Vacutainer. Oxalates are anticoagulants which form insoluble complexes with calcium ions (see table 2-1 for a listing of important ions, their symbol and valence and table 2-2 for the periodic table of elements). Oxalates are available as the $Na^+$, $K^+$, $NH_4^+$, and $Li^+$ salt. The major disadvantage in the use of oxalates as an anticoagulant is that they cause water to diffuse from red blood cells (RBC) to the plasma. This plasma dilution may, in some cases, cause as much as a five percent decrease in the concentration of plasma analytes. The technician must avoid performing tests that are affected by this anticoagulant. Oxalates also inhibit several enzymes, including amylase, acid and alkaline phosphatase, and lactate dehydrogenase.

| Name | Symbol | Valence |
|---|---|---|
| Hydrogen | H | +1 |
| Sodium | Na | +2 |
| Aluminum | Al | +3 |
| Silver | Ag | +1 |
| Zinc | Zn | +2 |
| Copper (I) or Cuprous | Cu | +1 |
| Copper (II) or Cupric | Cu | +2 |
| Mercury (I) or Mercurous | Hg | +1 |
| Mercury (II) or Mercuric | Hg | +2 |
| Iron (II) or Ferrous | Fe | +2 |
| Iron (III) or Ferric | Fe | +3 |
| Fluoride | F | -1 |
| Iodine | I | -1 |
| Chlorine | Cl | -1 |
| Bromine | Br | -1 |
| Oxide | O | -2 |
| Sulfide | S | -2 |

| Name of Complex Ion | Symbol | Valence |
|---|---|---|
| Ammonium | $NH_4$ | +1 |
| Hydroxide | OH | -1 |
| Nitrate | $NO_3$ | -1 |
| Nitrite | $NO_2$ | -1 |
| Sulfite | $SO_3$ | -2 |
| Sulfate | $SO_4$ | -2 |
| Carbonate | $CO_3$ | -2 |
| Bicarbonate | $HCO_3$ | -1 |
| Bisulfate | $HSO_4$ | -1 |
| Phosphate | $PO_4$ | -3 |
| Monohydrogen Phosphate | $HPO_4$ | -2 |
| Dihydrogen Phosphate | $H_2PO_4$ | -1 |
| Cyanide | CN | -1 |

Table 2-1. List of important ions.

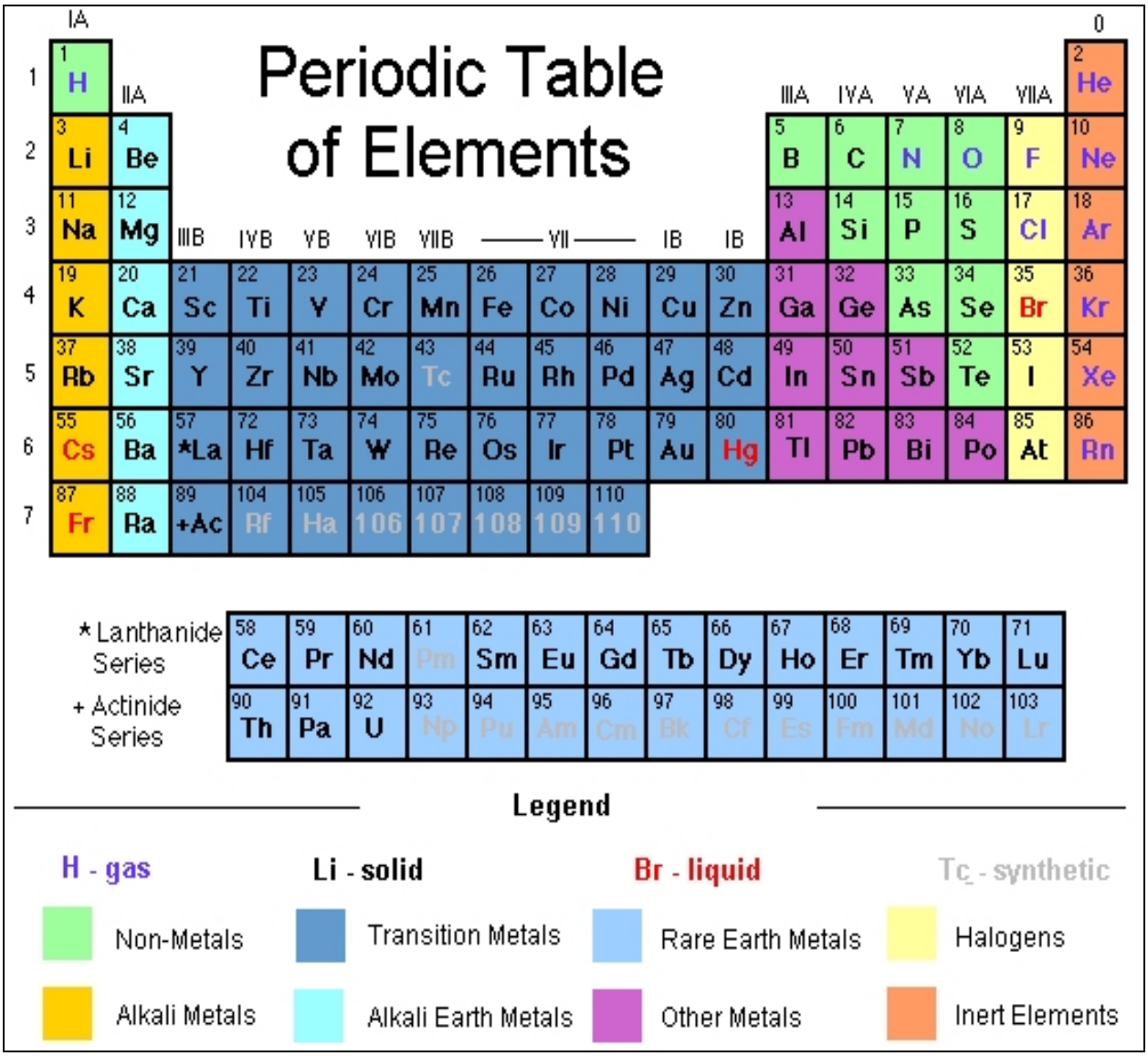



Table 2-2. Periodic table of elements.

(4) Fluoride--grey top Vacutainer. Sodium fluoride is most commonly used when determining glucose concentrations (see table 2-3 for additional preparation and use of some of the anticoagulants). It functions as a weak anticoagulant by virtue of its ability to precipitate calcium ions as $CaF_2$. It also serves as a chemical preservative by inhibiting the enzymes required for glycolysis (glucose utilization by RBCs). This anticoagulant is an inhibitor of other enzymes, including urease, an enzyme used for some blood urea nitrogen methods.

(5) Other anticoagulants. Sodium citrate (blue top vacutainer) is seldom used in clinical chemistry since it produces a significant water shift. It is widely used for coagulation studies.

| Anticoagulant | Preparation of Tubes (for 10 ml blood) | Remarks |
|---|---|---|
| Ethylenediamine-tetracetic acid, disodium or dipotassium salts. | Prepare a 0.1% aqueous solution. Dispense in tubes and dry at room temperature. Amount per tube is based on: 1 mg of salt per ml of blood. For a 10 ml. collecting tube use 0.1 ml. | The potassium salt is more soluble and is recommended. Do not use in sodium, potassium, or prothrombin time determinations. |
| Heparin, sodium | Dry reagent: prepare 1% aqueous solution. Use 0.2 ml. of this solution per 10 ml. collection tube. Liquid reagent (10 mg per ml. ampules): use 0.2 ml. per 10 ml. collection tubes. Dry tubes in desiccator or at 37° C in incubator. Store dried tubes at low temperature. | Best overall choice but effect not permanent. Use for routine tests except sodium and prothrombin time determinations. |
| Lithium oxalate | Prepare a 1.5% W/V solution. Put 1 ml. per 10 ml. collection tube. Dry in incubator at 37° C. | Best oxalate to use. Do not use for electrolytes including calcium. Best for plasma uric acid test. |
| Sodium oxalate or potassium oxalate mixture. | Same as for lithium oxalate. | Do not use for calcium, sodium, or potassium tests. Na Salt used routinely in prothrombin tests. |
| Sodium fluoride and potassium oxalate mixture. | Dissolve 1 g. sodium fluoride and 3 g. of potassium oxalate in 100 ml. of water. Place 1 ml. per 10 ml. collection tube. Dry in incubator at 37° C. | Primarily used when samples of whole blood are to be mailed. Particularly useful for preservation of glucose concentration. Do not use in any kind of enzyme test. |

Table 2-3. Preparation and use of anticoagulants.

## 2-3. COLLECTION AND PRESERVATION OF SERUM

a. **Collection of Serum**. Serum contains all the plasma constituents except the protein, fibrinogen, which is removed during the clotting process. Serum is usually obtained using a red top vacutainer, grey or red top (serum separation tube (SST)) vacutainer or by transferring the blood from the collecting syringe to a chemically clean test tube, without the use of an anticoagulant. The blood is allowed to stand at room temperature for 20 to 30 minutes to permit the complete formation of a clot. Once the blood has clotted, an applicator stick is used to separate the clot from the side of the tube ("rimming" or "ringing" the clot). If the blood was collected in a SST vacutainer tube, the tube already contains a barrier material which separates the serum and cells upon centrifugation. The blood is centrifuged for 10 minutes at 2000 RPM and the serum (supernatant) is removed as soon as possible. It is important that at least 20 minutes be allowed for the formation of the clot prior to centrifugation to prevent the later formation of fibrin clots in the serum which can hinder analysis. On the other hand, to prevent changes in serum constituents, the serum should be separated from the clot as soon as possible. As a rule of thumb, serum should not be left on the clot for more than 60 minutes. When collecting blood for the determination of $CO_2$ ($HCO_3$), the blood must remain capped to prevent loss of $CO_2$. Even with extreme care, hemolysis does occur and the technician must note on the laboratory request slip that the specimen was hemolyzed. Since hemoglobin interferes directly by inhibiting enzymes (lipase) and yields significant amounts of colors (spectro-photometric procedures), it gives inaccurate results because the level of some substances is higher in the RBC than in the serum.

NOTE: To prevent hemolysis when transferring blood from the syringe to the test tube, remove the needle and slowly expel the blood from the syringe. Once the blood has clotted, an applicator stick is carefully used to separate the clot from the side of the test tube ("rimming" or "ringing"). Some laboratories use glass beads (placed on top of the clot) or some other commercially available means to ensure that the clot is completely removed from the serum during centrifugation. The specimen is centrifuged for approximately 10 minutes at 2000 rpm after which the serum (supernatant) is removed and transferred to a chemically clean test tube to await analysis.

b. **Preservation of Blood Specimens**. Tests performed on serum or plasma should be carried out as soon as possible to prevent changes in chemical constituents. However, if analysis must be delayed, the sample may be refrigerated (usually) for up to 24 hours after the technician has separated the serum or plasma from the cells. If longer periods of time are encountered, the sample should be frozen. Some analytes are labile in cold temperatures (e.g., lactate dehydrogenase), therefore, aliquots of the sample need to be kept at room temperature. Other analytes will degrade when exposed to light (e.g., bilirubin, carotene) and must be protected. If the sample is refrigerated or frozen, it must be well mixed and at room temperature before the

technician attempts to perform the analysis. This is only a general guideline for treatment of blood specimens that cannot be immediately analyzed. The only suitable method of preservation is to read the instructions that correspond to the methodology employed.

## 2-4. COLLECTION AND PRESERVATION OF CEREBROSPINAL FLUID

The difficulties involved in obtaining spinal fluid by lumbar puncture make it imperative that the technician treat the spinal fluid sample with extreme care. If at all possible, the specimen should be collected in three chemically clean, sterile tubes by the physician. The three tubes should be numbered from 1 to 3 in the order in which they were obtained. Each tube should contain from 2 to 4 ml of spinal fluid. Tube number 1 is reserved for chemical analysis and serological testing; however, it is also the tube which may contain blood from trauma. Tube number 2 is used for cell counts. Tube number 3 is used for bacteriological testing. Any spinal fluid in excess of the quantity required may be used for chemical analysis. It is essential that the chemical tests be conducted as soon as possible after obtaining the specimen. The concentration of glucose, in particular, is altered with time. If the specimen cannot be analyzed immediately, it may be refrigerated after the contaminating cells are removed.

**WARNING**

ALL BIOLOGICAL FLUIDS SUCH AS CSF, BLOOD, AND SPUTUM SHOULD BE CONSIDERED TO CONTAIN PATHOGENIC ORGANISMS AND BE TREATED AS AN INFECTIOUS MATERIAL. EVEN IF THE CONTAINER OF A CONTROL OR PATIENT SPECIMEN DOES NOT CONTAIN ANY OF THESE MICROBES, YOU ARE TO HANDLE THEM WITH EXTREME CARE AS IF THEY DO.

**WARNING**

NEVER PIPETTE BY MOUTH TO AVOID EXPOSURE TO HIV, HEPATITIS B, OR OTHER INFECTIOUS MATERIALS AND CORROSIVE OR TOXIC AGENTS. YOU MUST WEAR PROTECTIVE GLOVES, APRONS, AND EYE PROTECTION, AND WASH HANDS FREQUENTLY.

## 2-5. COLLECTION AND PRESERVATION OF SEROUS FLUID

Serous fluid is obtained by a physician from the pleural, pericardial, and peritoneal cavities. It resembles serum (thin and watery) and produces or contains serum. Usually the physician collects about 20 ml in a chemically clean, sterile test tube containing heparin. Serous fluid is examined chemically for the following chemical constituents: protein, cholesterol, lactate dehydrogenase, glucose, and amylase. This fluid also must be handled with extreme care since it may contain pathological microorganisms. If this specimen cannot be tested immediately, it may be refrigerated or frozen.

## 2-6. COLLECTION AND PRESERVATION OF SYNOVIAL FLUID

Synovial fluid is obtained by the physician from the joint and tendon spaces. Two to 5 ml specimens are collected in chemically clean, sterile tubes containing heparin. The fluid is tested for the following chemical constituents: glucose (fasting), total protein, protein electrophoresis, immunodiffusion, alkaline phosphatase and acid phosphatase. If this specimen cannot be tested immediately, it should be refrigerated or frozen.

## 2-7. COLLECTION AND PRESERVATION OF AMNIOTIC FLUID

There are a number of reasons amniotic fluid may be collected. They may include chromosomal studies, inborn errors in metabolism, and most often used in the verification of fetal lung maturity. The spectrophotometric measurement of bilirubin in amniotic fluid is used to evaluate hemolytic disease in the fetus (in utero). Approximately 10 ml of fluid is collected by the physician and immediately placed in a dark brown container to prevent photodegradation of bilirubin. The fluid must be sent to the laboratory immediately, where it is inspected for blood contamination, centrifuged to remove turbidity, and analyzed. If testing cannot begin immediately, the specimen can be refrigerated for up to 24 hours or frozen if a longer period of time is required to complete the testing.

**CAUTION**: Avoid direct contact with light sources AT ALL TIMES since bilirubin is destroyed by ultraviolet light.

## 2-8. COLLECTION OF URINE

There are numerous methods for the collection of urine. Regardless of the method used, the technician must remember that urinary constituents are not stable. A chemically clean and (preferably) sterile container is used for the collection of all urine specimens. If the specimen is not to be analyzed immediately, it must be refrigerated or frozen (see table 2-4).

a. **Random Specimen**. A random urine specimen is usually used for screening and can be collected any time. It may have a low concentration of solutes, because it is subject to variable dilution.

NOTE: Because this urine specimen is subject to variable dilution, it may have low concentration of solutes.

b. **First Morning Void**. As a result of the day to day consistency of the first morning void, it is preferred for routine urine examinations. This urine is more concentrated and acidic resulting in formed elements that are in higher concentration and stability. Catherization or "clean catch" specimens may be required, especially for microbiological testing.

| NAME OF SPECIMEN | QUANTITY | REASON FOR COLLECTION | REMARKS |
|---|---|---|---|
| Serum | Clot | Test for healthiness | Separate serum from clot |
| Cerebrospinal fluid | 1-3 tubes 2-4 ml | Chemical, serology, bacteriological testing, cell counting | NOTE: Concentration of glucose may change |
| Serous fluid | 20 ml | Protein, cholesterol, lactate dehydrogenase, glucose, and amylase | CAUTION: May contain pathological microorganisms |
| Synovial fluid | 2 tubes 5 ml | Glucose, total protein, protein electrophoresis, immunodiffusion, alkaline phosphatase, acid phosphatase | Refrigerate or freeze |
| Amniotic fluid | 10 ml | Chromosome, metabolism, bilirubin | Check for blood contamination |
| Urine | Sufficient | Diurnal variations | Refrigerate or freeze |

Table 2-4. Collection and preservation of specimen.

c. **Postprandial Specimen**. This urine specimen is collected exactly 2 hours after the patient has completed a meal. This specimen has the greatest probability of containing glucose or protein.

d. **Afternoon Specimen**. The afternoon specimen is usually required for the detection of urobilinogen. The optimum time has been found to be within 1400 and 1600 hours.

e. **24-Hour Specimen**. In clinical chemistry, the 24-hour urine specimen is required for most chemical testing because of diurnal variations. The 24-hour specimen requires the cooperation of the patient, physician, and laboratory. The patient is usually directed to the laboratory to obtain the container and given directions concerning the proper method of collection. It is important that the laboratory technician give the proper instructions and ensure that the patient understands them. The method of collecting a 24-hour urine is as follows.

(1) Give the patient a chemically clean container that contains the proper preservative (if any).

(2) This container must be capable of containing 3 to 4 liters. Write the patients name and any special information (e.g., "CAUTION: CONTAINS 6 Eq/L HCl") on the outside of the container.

(3) Instruct the patient to void (not in the container) in the morning of the first day and discard it.

**CAUTION**: DO NOT void this specimen into the collection container.

(4) Instruct the patient to void all subsequent specimens into the container during the next 24 hours. Exactly 24 hours after the initial voiding (the first morning specimen), the final specimen is collected in the container; for example, 0730 hours of the second morning. The patient must write this time on the collection container.

(5) The specimen should be refrigerated, if possible, during the collection period.

f. **Day and Night Specimens**. There are two modifications of the 24-hour urine collection: the day specimen and the night specimen.

(1) For the day specimen, have the patient empty his bladder before breakfast and discard it; for example, at 0730 hours, and collect all specimens for the next 12 hours.

NOTE: The evening meal must be eaten at least 3 hours before the final specimen is collected. Exactly 12 hours later, the patient voids and collects the final specimen.

(2) For the night specimen, this is also a 12-hour collection. Have the patient empty his bladder in the evening, for example, at 2000 hours, and collect all urine specimens during the next 12 hours.

NOTE: The first specimen is discarded and the test should start at least 3 hours after the patient has finished the evening meal. Exactly 12 hours later and before the patient eats breakfast, he empties his bladder and collects the final specimen.

g. **Common Sources of Error.** Some of the commonly occurring sources of error in collecting 24-hour urines are inadequate preservatives (if required), loss of voided specimens, and collecting the "first" morning specimen and discarding the second morning specimen. After the specimen has arrived in the laboratory, the technician is required to measure the total 24-hour volume, saving an aliquot for analysis. Three technician errors commonly occur at this point:

(1) Careless measuring and/or recording of the total volume.

(2) Inadequate mixing of the total specimen before an aliquot is taken.

(3) Insufficient volume of urine aliquot for repeat testing. Some laboratories automatically perform a urinary creatinine determination on all 24-hour urine to check for errors in collection.

## 2-9. PRESERVATION OF URINE

The method for preservation of urine samples, especially 24-hour specimens, will depend upon the test(s) being performed. Constantly keep in mind that not all chemical tests can be performed on the same 24-hour urine specimen. There are chemical interferences and/or destruction of certain chemical components by some preservatives. Table 2-5 shows a typical laboratory chart that denotes the type of preservative and the tests that can be performed on the same 24-hour urine.

NOTE: This is a guide and may not apply to all methodologies employed.

a. **Refrigeration**. Refrigeration of specimens is a good practice, especially if no preservative has been added to prevent bacterial growth and preserve formed elements.

b. **Preservatives.** Some of the common preservatives used are given below.

(1) Toluene or thymol. Toluene or thymol are used to inhibit growth of aerobic bacteria. When toluene is used, the sample must be pipetted from below the toluene layer.

(2) Formalin (40% formaldehyde). This formaldehyde is a good preservative for formed elements; however, it can interfere with certain chemical tests.

(3) HCl. Hydrochloric acid is used to inhibit bacterial growth by lowering urine pH. This preservative is usually used for special chemical procedures like VMA.

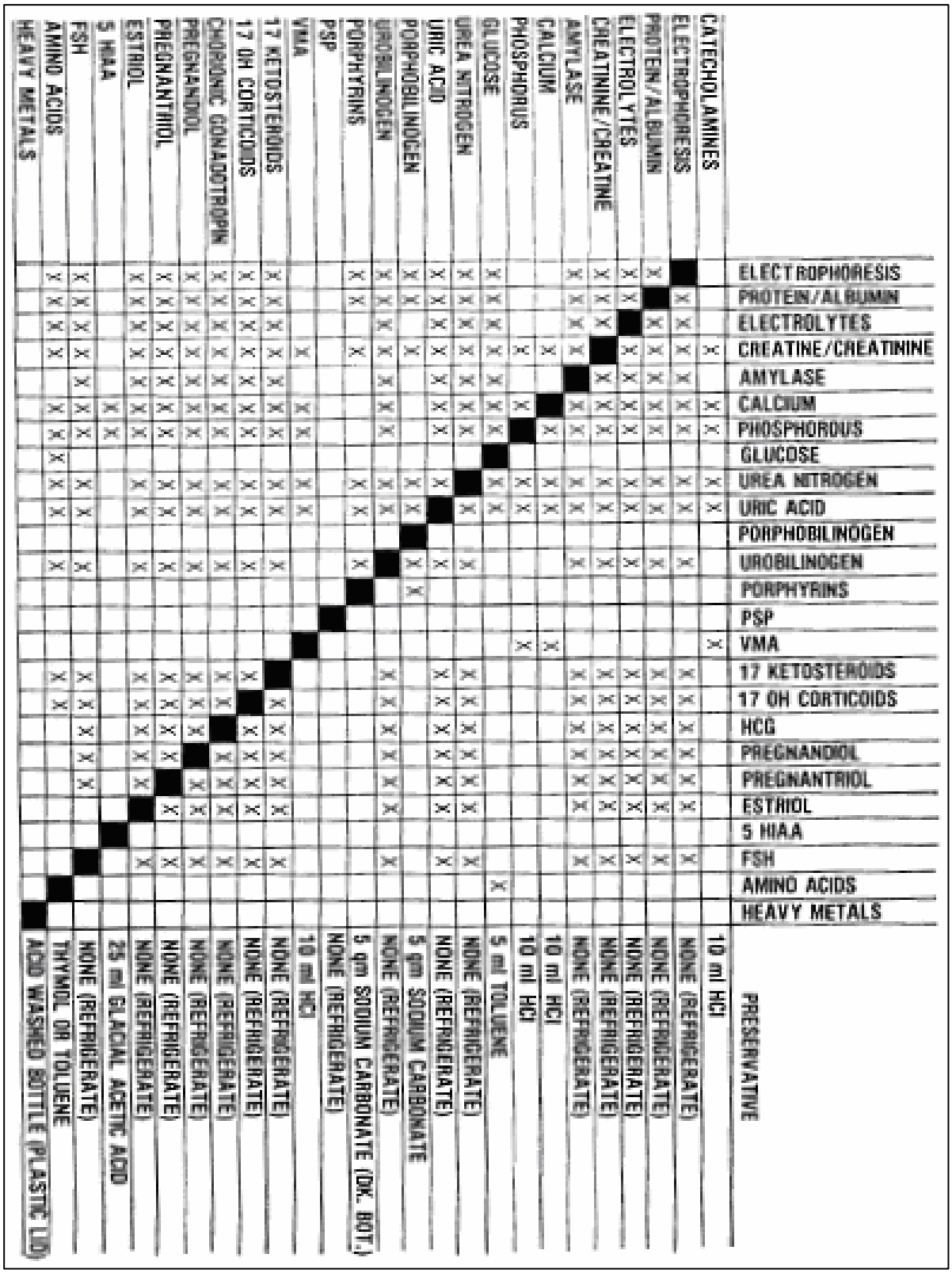



Table 2-5. 24-hour urine collection reference chart.

(4) Sodium carbonate. Sodium carbonate is used for the preservation of porphyrins. The change in pH will change the chemical integrity of steroids and other chemicals.

(5) Other chemicals. Other chemicals are used for special tests and it is up to the technician preparing the collection container to coordinate with the receiving lab as to the correct preservative to be used.

## 2-10. COLLECTION OF FECAL SPECIMENS

Since fecal specimens vary in quantity and quality, the chemical analysis is performed on 48- or 72-hour specimens. The 72-hour collection is recommended. Care must be taken to ensure the proper collection technique is used. Some laboratories may use a clean one gallon paint can, with lid, for the collection. During the 72-hour period, the patient is instructed to add each stool specimen to the container and to avoid depositing blood, urine, or other foreign material into the container. Before the technician gives the container to the patient, the container must be weighed ($W_0$) and recorded on the outside of the container. If a preservative is used, its weight must be included in the initial weight. When the patient returns the specimen to the laboratory, the weight of the fecal material is calculated as follows: $W_f = W_t - W_0$, where $W_f$ is the weight of the fecal material collected, and $W_t$ is the weight of the fecal material and collection container. Before an aliquot is taken for analysis, the fecal material must be thoroughly mixed to ensure that the sample being analyzed is representative of the 72-hour collection. Technicians must be extremely careful in handling fecal material to prevent contamination of themselves, the laboratory area, or alteration of the sample itself.

# Section II. CRITERIA FOR COLLECTION AND ACCEPTANCE OF SPECIMENS

## 2-11. COLLECTION VARIABLES

Test values can change within one day or day to day due to variables that may or may not be controlled by the collecting technician. By standardizing specimen collection practices, most, but not all, of the variables can be minimized. Understanding the effects of these variables will allow the technician to collect a specimen that is best representative of the patient's status.

a. **Diurnal Variation.** Some analytes will naturally vary in concentration during particular periods of the day. The time of collection must be known for the proper evaluation of the reported result. Examples of analytes which have a diurnal variation include cortisol, iron, estriol, glucose, renin, and triglycerides.

b. **Posture.** The posture of the patient will have an effect on those analytes that are protein or protein-bound. When you stand, the plasma volume is less than when sitting, and it must be compensated with an increase in plasma proteins. Enzymes, bilirubin, iron, calcium, total protein, and albumin will vary in concentration depending upon the patient's posture at the time of collection.

c. **Stasis**. Keeping the tourniquet tied on the patient's arm for a prolonged period of time will result in the elevation of many analytes.

d. **Hemolysis**. Lysing of the red blood cells during collection or processing will result in many different types of errors. These include falsely elevated serum/plasma analyte concentrations for those analytes found in high concentration within RBCs, falsely decreased results for some analytes diluted by hemolysis, and interference by hemoglobin in certain spectrophotometric methods.

e. **Preservatives or Anticoagulants.** It is necessary to draw blood in the Vacutainer tubes in an order so that the preservatives or anticoagulants in one tube will not contaminate the next one. For example, if an EDTA tube is drawn before a red top tube, it may contaminate the red top inhibiting certain enzymes to be analyzed.

f. **Diet.** Fasting patients will have different results for most tests than when they do not fast. Caffeine will cause a slight increase in glucose and catecholamines.

g. **Stress.** Patients who have recently exercised, are ill, or are anxious about the collection procedure will have varied test results.

## 2-12. CRITERIA FOR AN UNACCEPTABLE SAMPLE

The clinical laboratory should have established criteria for the rejection of submitted specimens for testing. Some means of documentation of rejected samples should be kept and monitored to ensure patterns do not become routine and allow for corrections to be made to reduce future problems.

a. **Improper Sample Identification.** One of the most often seen examples is the difference between the name on the lab slip and the sample container. The safest procedure to ensure correct sample and patient matching is to redraw the sample.

b. **Improper Collection Tube.** Generally, serum is the sample of choice for most clinical chemistry analyses. Although it is not uncommon to see the anticoagulant heparin used since it is least likely to affect chemistry procedures, it is method dependent.

c. **Inadequate Blood Volume in Collection Tube**. Although it is thought to be a criteria only with anticoagulated specimen tubes, "short" draws may also affect serum by causing hemolysis. This may interfere with testing procedures.

d. **Hemolysis**. The degree of interference depends upon the extent of hemolysis, analyte concentration, and method of testing. The following analytes can be significantly affected:

(1) Potassium.

(2) Lactate dehydrogenase.

(3) Acid phosphatase.

(4) Creatine phosphokinase.

(5) Iron.

(6) Magnesium.

(7) Bilirubin (may cause a negative interference).

## Section III. SHIPMENT OF SPECIMENS

### 2-13. SHIPMENT OF SPECIMENS

a. **Shipment of Specimens**. When specimens are to be shipped, the sending and receiving laboratories must have effective channels of communication. The receiving laboratory should furnish a current SOP to all sending laboratories that states:

**(**1) Amount of specimen required for the test.

(2) Type of specimen required.

(3) Preservative (if required).

(4) Method of shipment (i.e., on ice, frozen, etc.).

(5) Special instructions.

b. **Common Shipment Problems**. Common problems encountered in shipping specimens that are unacceptable include poor packing of specimens resulting in broken tubes or spilled contents; improper labeling of specimens, wrong specimen, wrong preservative used, hemolyzed specimens, and putrefied specimens resulting from the lack of freezing or icing. The delay between the time the specimen is collected and the results returned to the sending lab may be lengthy; however, errors by the sending lab simply intensify this problem. If there is any question concerning the shipment of specimens, contact the receiving laboratory before processing the specimen.

c. **Improper Transportation.** Some samples can be severely affected by improper transport. For example, blood gases, ammonia, and lactic acid require transport to the laboratory on ice and within a specified time.

d. **Other Interferents**. This may include icteric serum, lipemia, turbidity, and drugs.

## 2-14. CHAIN OF CUSTODY

a. **Medico-legal Implications**. Specimens (blood alcohols, drugs, or forensic specimens) whose results will have medico-legal implications require handling in an appropriate forensic manner so that data will be recognized in a court of law. All processing steps (which include collection, transport, storage, and testing) must be documented to ensure that there has been no tampering by interested parties. It must be ensured that the specimen belongs to the appropriate individual and the results are reported accurately.

b. **Chain of Custody**. To accomplish this, a "chain of custody" document is used, DD Form 1323, Toxicological Examination Request and Report. It establishes a chain of custody for all specimens, documenting the type of specimen(s) and signatures of all individuals who have handled the specimen during the collection and analysis. The form and the specimen must be secured at all times until it is released to authorized personnel. The proper procedures to follow while filling out DD Form 1323 are covered in a different subcourse, MD0866 Toxicology and Therapeutic Drug Monitoring.

c. **Storage and Retention of Records**. Specimens should be properly stored (usually 4$^{\circ}$C) until time to transport and must be adequately sealed at all times to ensure its integrity. Quality control data and other information pertaining to the sample should be kept available. If the specimen is to be saved, the same chain of custody procedures must be followed.

**Continue with Exercises**

## EXERCISES, LESSON 2

**INSTRUCTIONS:** Answer the following exercises by marking the lettered response that best answers the question, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Chemical integrity of a specimen consists of:

   a. Type of specimen.

   b. Type of anticoagulant used, it any.

   c. Amount of specimen required for the specific test.

   d. All of the above.

2. What is the clinician's only reason for determining the results of a blood specimen test?

   a. To maintain credibility and reputation.

   b. To determine how the results reflect the condition of the patient.

   c. To give a high carbohydrate drink if needed.

   d. To determine if turbidity from increased chylomicrons may interfere with colorimetric spectrophotometric procedures.

3. Which statement is the definition of a fasting specimen?

    a. A specimen which is drawn at least 2 hours after the patient has completed a meal.

    b. A specimen which is drawn from a patient who has been without food for approximately 4 hours.

    c. A specimen which is drawn from a patient who has been without food for approximately 8 hours.

    d. A specimen which is drawn from a patient who is on a sodium and fat restricted diet.

4. A fasting specimen:

    a. Has little diagnostic value except to establish a baseline to follow the course of treatment.

    b. Is by far the most common type of specimen requested.

    c. Is drawn exactly two hours after a patient has completed a meal or been given a high carbohydrate drink (Glucola).

    d. Does not affect the testing for phosphate concentrations because they will be decreased.

5. A random specimen:

    a. Has little diagnostic value except to establish a baseline to follow the course of treatment.

    b. Is drawn exactly two hours after a patient has completed a meal or been given a high carbohydrate drink (Glucola).

    c. Affects the testing of phosphate concentrations because the concentrations will be decreased.

    d. Affects the turbidity. The turbidity from increased chylomicrons may interfere with colorimetric spectrophotometric procedures.

6. Which statement is the definition of a two hour postprandial specimen?

    a. A specimen which is drawn 2 hours after a bowel movement.

    b. A specimen which is drawn exactly 2 hours after the administration of a glucose tolerance test.

    c. A specimen which is drawn 2 hours after the patient has swallowed 50 ml of 0.9 sodium chloride solution.

    d. A specimen which is drawn exactly 2 hours after a patient has eaten a meal.

7. The two hour postprandial specimen:

    a. Affects the turbidity. The turbidity from increased chylomicrons may interfere with colorimetric spectrophotometric procedures.

    b. Requires that the patient fast so that phosphate concentrations will increase.

    c. May require the patient to ingest a high carbohydrate drink like (Glucola).

    d. Requires fasting before the specimen is drawn so that the glucose and triglyceride concentrations will be decreased.

8. Select the anticoagulant which must NOT be used when collecting blood for a phosphate determination?

    a. Heparin.

    b. EDTA.

    c. Sodium oxalate.

    e. Fluoride.

9. Which anticoagulant will interfere least with most chemistry tests?

    a. Heparin.

    b. EDTA.

    c. Potassium oxalate.

    d. Sodium fluoride.

10. EDTA is an example of a chelating anticoagulant. What is a chelator?

    a. It is a substance that interferes with glucose.

    b. A substance that binds metal ions.

    c. This substance kills the cellular components of the blood.

    d. EDTA is used when calcium determinations are required or the test that corresponds to the salt being used.

11. Which statement about EDTA is correct?

    a. This anticoagulant is available as disodium and dipotassium salts, the former being preferred because of its greater solubility in aqueous solutions.

    b. It has been reported that EDTA does not interfere with glucose, urea, or creatinine determinations; but, it will reduce prothrombin time.

    c. It has been reported that EDTA does interfere with glucose, urea, or creatinine determinations; but, it will reduce prothrombin time.

    d. It preserves the cellular components of the blood.

    e. b and d.

    f. EDTA can be used when calcium determinations are required or the test that corresponds to the salt being used.

12. What color coded Vacutainer tube contains the anticoagulant EDTA?

    a. Red.

    b. Lavender.

    c. Green.

    d. Black.

13. The oxalates, with the black top Vacutainer:

    a. Are insoluble with calcium ions.

    b. Are not available as the $Na^+$, $K^+$, $NH_4^+$, and $Li^+$ salt.

    c. Function as a weak anticoagulant by virtue of its ability to precipitate calcium ions as $CaF_2$.

    d. Produce a significant water shift.

14. What is a major disadvantage of oxalates?

    a. They increase several enzymes, including amylase, acid and alkaline phosphatase, and lactate dehydrogenase activity.

    b. As an anticoagulant, they cause water to diffuse from RBCs to the plasma. This dilution may cause as much as a 5% decrease in the concentration of plasma analytes.

    c. Function as a weak anticoagulant by virtue of their ability to precipitate calcium ions as $CaF_2$.

    d. Produce a significant water shift.

15. Fluoride-gray top Vacutainers:

    a. Serve as a chemical preservative to inhibit enzymes required for glycolysis and other enzymes, including urease.

    b. Are the best oxalates to use but not with electrolytes that include calcium.

    c. Are to be dispensed in tubes and dried at room temperature.

    d. Function as a weak anticoagulant by virtue of their ability to precipitate calcium ions as $CaF_2$.

    e. a and d.

16. Regardless of the method used to collect blood, when and how must the technician mix the blood specimen with the anticoagulant?

    a. After 5 minutes by gentle centrifuge.

    b. After 6 minutes, shake it.

    c. Immediately by gentle inversion.

    d. Immediately by dilution.

17. What is removed once the blood and anticoagulant are mixed and the tube is then centrifuged at approximately 2000 RPM for 10 minutes?

    a. Prothrombin.

    b. Plasma (supernatant fluid).

    c. Anticoagulant.

    d. Oxalate.

18. Lithium oxalate is:

    a. Best used for the plasma uric acid test.

    b. A liquid reagent. It is to be used at 0.2 ml per 10 ml collection tube.

    c. Used for routine tests except sodium and prothrombin time determinations.

    d. Prepared the same as was sodium oxalate.

    e. a and d.

19. Which anticoagulant is the most commonly used when determining glucose concentrations?

    a. Heparin.

    b. EDTA.

    c. Oxalate.

    d. Fluoride.

20. Using table 2-3, which anticoagulant is used primarily when samples of whole blood is to be mailed?

    a. Lithium oxalate.

    b. Heparin.

    c. EDTA.

    d. Sodium fluoride and potassium oxalate.

21. Serum differs from plasma in that serum does NOT contain:

    a. Glucose.

    b. Globulin.

    c. Albumin.

    d. Fibrinogen.

22. When separating serum, the term "rimming" or "ringing" is used. What does this term mean?

    a. Combining serum and blood.

    b. Removing the needle slowly from the test tube and expelling the blood from the syringe.

    c. Applying an applicator stick to carefully separate the clot from the side of the test tube.

    d. Using glass beads and placing them on top of the clot.

    e. Commercially removing the partial clot from the serum during centrifugation.

23. Why is it important to allow the blood to stand before centrifugation and for at least how many minutes?

    a. To allow the complete formation of fibrin clots; 20-30.

    b. To enhance the process; 19.

    c. To prevent changes in the serum constituents; 12.

    d. None of the above; 10.

24. Why should sera be separated from the blood clot as soon as possible?

    a. To prevent the formation of fibrin clots.

    b. To enhance the process.

    c. To prevent changes in the serum constituents.

    d. None of the above.

25. When collecting blood to determine the $CO_2$ ($HCO_3^-$), the blood must remain capped to prevent loss of $CO_2$. Even with extreme care, how and why can hemoglobin interfere and cause inaccurate serum results?

    a. It doesn't homologize causing large clots and poor results.

    b. It interferes directly by inhibiting enzymes (lipase) and yields significant amounts of colors (spectrophotometric procedures) and results are inaccurate because the level of some substances is higher in the RBC than in the serum.

    c. It indirectly inhibits enzymes (lipase) and yields significant amounts of colors (spectrophotometric procedures) and results are inaccurate because the level of some substances is higher in the RBC than in the serum.

    d. It yields small amounts of colors (spectro-photometric procedures) causing inaccuracy because the level of some substances is higher in the RBC than in the serum.

26. When testing must be delayed, for how many hours may separated serum or plasma sample be preserved by refrigeration?

    a. 12.

    b. 14.

    c. 21.

    d. 24.

27. The collection of cerebrospinal fluid must be handled very carefully. Which set of instructions is correct?

    a. The physician uses 3 chemically clean, sterile tubes, numbered from 1 to 3 in the order in which they were obtained, and collects in each tube from 2 to 4 ml of spinal fluid.

    b. The technician uses chemically clean tubes and each tube should contain from 2 to 4 ml of spinal fluid.

    c. a and b.

    d. The physician uses 3 chemically clean, sterile tubes and each tube should contain from 3 to 5 ml of spinal fluid.

28. Which statement about the collection/preservation of cerebrospinal fluid is correct?

    a. Spinal fluid in excess of the quantity needed may be used for chemical analysis.

    b. Cerebrospinal fluid does not present any problems with microbial contamination since it is always sterile.

    c. Cerebrospinal fluid can be collected easily and quickly.

    d. If the specimen cannot be analyzed immediately, it should be freeze-dried.

29. As a technologist, for the analysis of cerebrospinal fluid, you must know the order in which each aliquot of CSF was dispense into each of the 3 tubes before you can analyze the fluid. Which statement is correct?

    a. Tube numbered: 1 is used for bacteriological testing; 2 is reserved for chemical analysis and serological testing, however, it is also the tube which may contain blood from trauma; 3 is for cell counts.

    b. Tube numbered: 1 is reserved for chemical analysis and serological testing, however, it is also the tube which may contain blood from trauma; 2 is for cell counts; 3 is used for bacteriological testing.

    c. Tube numbered: 1 is for cell counts; 2 is reserved for chemical analysis and serological testing, however, it is also the tube which may contain blood from trauma; 3 is used for bacteriological testing.

30. Cerebrospinal fluid may contain microorganisms which are pathological; therefore, you would:

    a. Pipet cerebrospinal fluid specimen by mouth.

    b. Not pipet any cerebrospinal fluid specimen by mouth.

    c. Not be concerned.

    d. Pipet as usual.

31. Serous fluid is also handled very carefully because it may contain pathological microorganisms. If not tested immediately, it may be refrigerated or frozen. What chemical constituents do you examine?

    a. Glucose and prothrombin.

    b. Protein and amino acids.

    c. Protein, cholesterol, lactate dehydrogenase, glucose, and amylase.

    d. None of the above.

32. From which location does a physician obtain synovial fluid and for which chemical constituent can you test for only if the patient has been fasting?

    a. Tip of middle finger; alkaline.

    b. Arm; protein electrophoresis.

    c. Gluteus maximus; total protein.

    d. Joint and tendon spaces; glucose.

33. What is it that must be done immediately to amniotic fluid just collected to prevent photodegradation of the bilirubin?

    a. Place it in a dark brown container.

    b. Put it in the sunlight.

    c. Place it next to a 150 watt lamp.

    d. Put it in a petri dish.

    e. None of the above.

34. The first morning void:

    a. May have a low concentration of solutes because it is subject to variable dilution.

    b. Is more concentrated and acidic, resulting in formed elements that are in higher concentration and stability.

    c. Has the greatest probability of containing glucose or protein.

    d. Is usually required for the detection of urobilinogen and collected between 1400 and 1600 hours.

35. With the 24-hour specimen collection of urine:

    a. Give the patient a chemically clean container that contains the proper preservative.

    b. Instruct the patient to void in the morning of the first day and discard it.

    c. Instruct the patient to void all subsequent specimens into the container during the next 24 hours.

    d. And, exactly 24 hours after the 0730 hours of initial voiding of the first morning specimen, have the patient collect the final specimen in the container at 0530 hours.

    e. All of the above.

    f. a, b, and c.

36. When modification is needed for the 24-hour urine collection, for the night specimen:

    a. Have the patient eat her evening meal at least 2 hours before the final specimen is collected.

    b. Have the patient empty her bladder at 2300 hours and collect all urine specimens during the next 9 hours.

    c. Discard the second specimen and start the test at least 3 hours after the patient has finished the evening meal.

    d. Exactly 12 hours later and before the patient eats breakfast, have her empty her bladder and collect the final specimen.

37. Which is a common error in the collection of urine?

    a. Collecting the "second" morning specimen and discarding the first morning specimen.

    b. Inadequate mixing of the total specimen before an aliquot is taken.

    c. Carefully measuring and/or recording of the total volume.

    d. Using adequate preservatives.

38. How many tubes are needed to collect synovial fluid and how many ml per tube?

    a. 1 tube; 10 ml.

    b. 1 tube; 15 ml.

    c. 2 tubes; 5 ml.

    d. 6 tubes; 2 ml.

39. Which statement best describes the proper procedure for the collection/ preservation of urine?

    a. It is recommended that the specimen not be capped in order that gases contained in the urine might be allowed to escape thus reducing the odor problem associated with urine.

    b. The specimen container must be filled to the top.

    c. Catherization or "clean catch" urine specimens may be required for microbiological examination.

    d. Refrigeration of urine samples is an unsound practice because particulate matter may be removed from solution.

40. During the collection of a 24-hour urine, which void should not be collected?

    a. The first morning void at the end of the timed period.

    b. Any urine voided immediately following a high carbohydrate meal.

    c. Any urine void which is not at least 100 ml in total volume.

    d. The first morning void at the beginning of the timed period.

41. As the NCOIC, what should you check to ensure that the medical laboratory specialist is preserving urine properly?

    a. Check the method of urine preserved with the type of urine test performed.

    b. Compare the urine tests conducted with the types of tests that could be performed on the same 24 urine specimen.

    c. Compare the preservatives used with the preservatives required to ensure chemical integrity for desired urine test results.

    d. All of the above.

42. Which is not a common urine preservative?

    a. HCl.

    b. Thymol.

    c. Heparin.

    d. Sodium carbonate.

43. Formaldehyde is:

    a. Used to inhibit growth of aerobic bacteria.

    b. A good preservative for formed elements; however, it can interfere with certain chemical tests.

    c. Used to inhibit bacterial growth by lowering urine pH.

    d. Used for the preservation of porphyrins.

44. Toluene is:

    a. Used to inhibit growth of aerobic bacteria.

    b. The cause for the change in the chemical integrity of steroids and other chemicals.

    c. Used to inhibit bacterial growth by lowering urine pH.

    d. Used for the preservation of porphyrins.

45. Which statement is correct in the collection of fecal specimens?

    a. Fecal specimens vary in quantity and quality so the chemical analysis should be performed on 36 or 72-hour specimens.

    b. Use any one of the many collection techniques.

    c. Have the patient add each stool specimen to the container but avoid depositing blood, urine, or other foreign material.

    d. Weigh the fecal matter and preservative but not the container.

46. Which statement is correct in the collection of fecal specimens?

    a. Preservatives should not be used.

    b. Before an aliquot is taken for analysis, the fecal material must be thoroughly separated to ensure that the sample being analyzed is representative of the 72-hour collection.

    c. After the technician gives the container to the patient, it must be only weighed ($W_0$).

    d. Technicians must be extremely careful in handling fecal material to prevent contamination of themselves, the laboratory area, or alteration of the sample itself.

47. Which is NOT a criterion for an unacceptable sample?

    a. Medico-legal implications.

    b. Improper collection tube.

    c. Hemolysis.

    d. Amount of blood in the collection tube.

48. Which collection variable is associated with different test results at particular times of the day?

    a. Diurnal variation.

    b. Hemolysis.

    c. Stress.

    d. Posture.

49. Which criteria would determine that a shipped specimen is unacceptable?

    a. In the sending laboratory's SOP.

    b. If the specimen tube was labeled improperly.

    c. When heparin is used in the collection of a plasma specimen.

    d. Red top Vacutainer tube is used for collecting a serum specimen.

50. Which statement about DD Form 1323 is true?

    a. Signatures of all who handled the specimen must be included.

    b. Establish a chain of custody for a specimen.

    c. It is used for medical or legal tests.

    d. All the above.

**Check Your Answers on Next Page**

**SOLUTIONS TO EXERCISES, LESSON 2**

1. d (para 2-1)
2. b (para 2-2)
3. c (para 2-2a(2))
4. b (para 2-2a(2))
5. a (para 2-2a(1))
6. d (para 2-2a(3))
7. c (para 2-2a(3))
8. a (para 2-2b(1))
9. a (para 2-2b(1))
10. b (para 2-2b(2))
11. e (para 2-2b(2))
12. b (para 2-2b(2))
13. a (para 2-2b(3))
14. b (para 2-2b(3))
15. e (para 2-2b(4))
16. c (para 2-2b)
17. b (paras 2-2b, 2-3a)
18. e (table 2-3)
19. d (para 2-2b(4), table 2-3)
20. d (table 2-3)
21. d (para 2-3a)
22. c (para 2-3a, NOTE)

23. a (para 2-3a, NOTE)

24. c (para 2-3a, NOTE)

25. b (para 2-3a, NOTE)

26. d (para 2-3b)

27. a (para 2-4)

28. a (para 2-4)

29. b (para 2-4)

30. b (para 2-4 **WARNING**)

31. c (para 2-5)

32. d (para 2-6)

33. a (para 2-7)

34. b (para 2-8b)

35. f (para 2-8e)

36. d (para 2-8f(2) NOTE)

37. b (para 2-8g)

38. c (para 2-6, table 2-4)

39. c (para 2-8b)

40. d (para 2-8e(3))

41. d (para 2-9)

42. c (para 2-9b)

43. b (para 2-9b(2))

44. a (para 2-9b(1))

45. c (para 2-10)

46. d (para 2-10)

47. a (para 2-12)

48. a (para 2-11a)

49. b (para 2-12a)

50. d (para 2-14b)

**End of Lesson 2**

## LESSON ASSIGNMENT

**LESSON 3** Measurement of Weights and Volumes.

**TEXT ASSIGNMENT** Paragraphs 3-1 through 3-15.

**LESSON OBJECTIVES** After completing this lesson, you should be able to:

3-1. Select the statement that best describes a specific type of balance.

3-2. Select the most appropriate balance used in a specific situation.

3-3. Select the statement that best describes the proper care and/or use of a balance.

3-4. Select the statement that best describes the sequence of steps for cleaning glassware.

3-5. Define "to deliver" (TD) glassware.

3-6. Define "to contain" (TC) glassware.

3-7. Select the statement that best states the glassware to be used to measure fluid when a detailed description of a fluid (i.e., type and/or volume) is known.

3-8. Select the statement that best describes a consideration involved in the selection/use of a pipette.

3-9. Select the statement that best describes the uses of volumetric glassware.

3-10. Select the statement that best describes an important factor in the use of burets.

**SUGGESTION** After studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

# LESSON 3

# MEASUREMENT OF WEIGHTS AND VOLUMES

## Section I. MEASUREMENT OF WEIGHTS

### 3-1. INTRODUCTION

a. **General**. Clinical laboratory technology has in recent years developed to such proportions that highly trained specialists are essential to support the physicians and pathologists in their daily activities. In the forefront of these advances is the field of clinical chemistry with its ever increasing responsibilities in practical applications to clinicians for their diagnosis and treatment of disease.

b. **Modern Methodology**. The development of new procedures on various body fluids, the advent of instrumentation and new interpretations of old tests combined with rapid, simple methods have made clinical chemistry along with the other aspects of the clinical laboratory, a continually challenging and diversified field.

### 3-2. BALANCES

a. In the modern clinical laboratory, measurements of mass are seldom performed. Reagents, standards, and controls come ready to use or simply need reconstituting. However, since measurement of mass is fundamental to every analysis, the use of some sort of balance is inevitable. It may be necessary to prepare drug standards from pure, authentic material. Fecal fats may be measured by gravimetric analysis. Of course, volumetric equipment is calibrated by measurement of mass.

b. The weight of a substance is a function of a property of all substances, mass, and the effect that gravity has on the mass. This relationship can be expressed by the equation: WEIGHT = MASS x GRAVITY. Mass is a measurement of an object's resistance to a change in motion. For example, a ping-pong ball moving at 1 cm/sec can be easily stopped by a gentle breeze; however, a bowling ball moving at the same speed is not. Therefore, the bowling ball has a greater mass than the ping-pong ball. To determine the mass of the substance, by a process known as weighing, the weight of the substance must be compared to the weight of a known **calibrated mass**. Two substances of equal weight and subject to the same gravitational force will have equal mass. This comparison is the process by which a balance operates. The gravitation force where the balance is located will affect both the unknown mass and the calibrated mass equally. Changing the location of the balance to places where the gravitational force differs will not affect the function of the balance, because the weight of the substance is always relative to the weight of the calibrated mass.

c. Although the classic form of the balance is greatly antiquated, modern balances (both mechanical and electronic) continue to apply the principle of equilibrium in a variety of ingenious ways. All balances require a vibration free location. The more sensitive balances need more protection not only from vibration but air currents that can disturb the equilibrium between the weighed object and weights. Also of importance is the cleanliness of the balance. Chemical substances should never be placed in direct contact with the weighing pans. Loose crystals or liquids with corrosive vapors should not be permitted to remain on or around the immediate area of the weighing pans. Good weighing technique at sensitivities under one gram calls for handling weights with forceps. Technicians need to handle weighed objects with proper utensils and avoid moisture, oils, or salts that their hands could pick up and deposit onto the weights or weighing pans.

## 3-3. PRINCIPLES OF WEIGHING

There are two principles of weighing--substitution and direct opposition.

a. **Substitution**. In weighing by substitution, weights are removed from the side of the balance to which the object to be weighed has been added to restore equilibrium.

(1) Single pan. In this method of weighing, a single pan is suspended from one position on one balance arm along with all of the **adjustable weights**. A stationary counterweight is attached to the arm opposite the first arm and on the other side of the fulcrum. With the pan empty, the instrument is balanced at a weight readout of zero. When the object is placed on the pan, the balance is upset, and weight must be removed from the heavier arm to restore balance. This is accomplished by lifting up and removing sufficient adjustable weights until the arms are balanced again.

(2) Double pan. The object weight is substituted for the removed weights. With the pan empty, the instrument is balanced at a weight readout of zero. When the object is placed on the pan, the balance is upset, and weight must be removed from the heavier arm to restore balance. This is accomplished by lifting up and removing sufficient adjustable weights until the arms are balanced again. In effect, the object weight is substituted for the removed weights. Therefore, the weight of the object is equal to the weight of the removed weights. Unlike the opposition **double pan** balances, substitution balances are read when the arm has come to rest on the single pan. This method is called **substitution**.

b. **Opposition.** In weighing by direct opposition (also called direct comparison), weights are added to one side of the beam to counterbalance the weight of the object on the other side. This is the most common approach.

(1) Double pan. This necessitates the use of two pans, one for the object being weighed and one for the weights. In these balances, the weights may be applied in two ways.

(a) First, separate weights can be placed on the pan opposite from the object until balance is reached.

(b) Secondly, by sliding weights, which rest on a beam or beams that parallel the arms, they can be moved to exert weight toward the pan used for the weights.

(2) Separate and sliding weights. Both the separate and sliding weights can be used together to exert the force necessary to balance the arms about the centered fulcrum. These balances use an oscillating rest-point operational technique; the speed of operation is fairly slow.

## 3-4. TYPES OF BALANCES

a. As previously mentioned, a balance may have one or two weighing pans. Double pan balances conform to the classic design with a single beam with arms of equal length. Standard weights are usually added manually to the right side pan to counter-balance the weight of the object on the other (see table 3-1). In single pan balances, the arms are of equal length. The object to be weighed is placed on the pan. A restoring force is applied **mechanically or electronically** to the other arm to return the beam to its null position. Double-and triple-beam balances are forms of the unequal arm balance. Each type of balance may have different models depending upon its fundamental construction and method of weighing (see figure 3-1). For example, an analytical balance may be either a substitution or an opposition balance. Most balances use a knife-edge fulcrum which varies in quality with the precision of the instrument.

b. Laboratory balances are mechanical and electronic in design. Among the classes of laboratory balances, these are generally recognized:

| TYPE | APPROXIMATE CAPACITY | REPEATABILITY/ PRECISION |
|---|---|---|
| Course | 2 kg | 0.1 g |
| Analytical | 100-200 g | 0.1 mg |
| Semi-micro | 75-100 g | 0.01 mg |
| Micro | 1-30 g | 1.0 mg |
| Ultra-micro | less than 5 g | 0.1 g |

Table 3-1. Classes of balances.

Figure 3-1. Types of balances.

## 3-5. MECHANICAL BALANCES

a. **Trip Balance, Double Pan Balance, or Equal-Arm Balance.** The trip balance consists of two pans of equal mass suspended from the ends of a beam that is supported at its center of gravity by a knife-edge fulcrum. The "trip balance" shown in figure 3-2 is a dual beam (two scales) model and its fulcrum is usually made of plastic or hardened steel and sometimes inexpensive quartz. This balance is probably the most common and is widely used. It is called the "general purpose balance." One scale, with a 0.1 gram rider, is calibrated from 0 to 10 grams. The second scale, with a 10 gram rider, is calibrated from 0 to 200 grams. Any weighing above the 200 gram range must be accomplished by using a separate weight set. The trip balance is used to weigh items that do not require a great deal of precision. The material to be weighed is placed on the left pan. The final weight is obtained by adjusting the position of a rider or small weight on an extension of the beam, called the balance arm bridge. The balance arm bridge is calibrated in 0.1 g increments up to a maximum of 200 g.

Figure 3-2. Trip balance.

b. **Single Pan Balance**. In addition to the trip or double pan balance, there are single pan balance styles that have two or three beams with sliding weights (see figure 3-3). They operate on the same principle as the double pan balances. The single pan balance is a modified trip balance with arms of equal length. The fulcrum is located close to the weighing pan. The right arm of the beam consists of two or three balance arm bridges that support counterbalancing weights. Trip balances are useful for weighing masses quickly when a weight to the nearest 0.1 g is satisfactory, as in the preparation of reagents such as strong bases and salts.

Figure 3-3. Single pan balance.

c. **Coarse Balance**. Coarse balances are used for weighing applications that do not require great precision. For example, these balances are used for weighing large amounts of chemical substances or for balancing centrifuge tubes where the precision required does not exceed 0.1 gram.

(1) Regular balance. Coarse balances generally have a capacity of two kilograms (2000 grams), although some styles of this type may have a smaller or greater capacity of weighing. The sensitivity of these balances is approximately 0.1 gram. Coarse balances can be used with moderate to rapid speed. The accuracy of these balances may vary as much as ± 0.5 gram since the construction of these balances is not always precise. In other words, even though 10.5 grams of a substance could be weighed, the true weight of the substance may be as much as 11.0 grams or as little as 10.0 grams.

(2) Top-loading balance. Top loading styles that are substitution models are also manufactured and are present in some clinical laboratories (see figure 3-4).


Figure 3-4. Top loading balance.

d. **Analytical Balances**. Analytical balances generally have a greater readability, precision, and accuracy than the coarse type of balance. This type of balance is widely used in clinical laboratories when greater precision is required when weighing certain substances. A true analytical balance has a precision of 0.0001 gram (0.1 milligram). Weighing capacities of analytical balances vary from 100 grams to 200 grams-depending upon the model of balance being used. Two models are in use today, the older two-pan opposition balance, such as the rider analytical balance (see figure 3-5), and the newer substitution single pan or top loader model. The styles of opposition analytical balances (double-pan) have sliding, "riding," or hanging "chain" weights that are adjustable for smaller increments of weights. The larger increments of weights are in the form of separate weights, which are stored in a special box when they are not being used. Both the opposition and the substitution analytical balances have their arms balanced about a knife edge fulcrum which is usually made of high-quality quartz which maintains a keen edge.

(1) Older two-pan opposition balance.

(a) Rider analytical balance. The Rider type used is a 1 milligram wire rider and is preformed to fit over the beam. The wire rider is fitted with a hole at the top so that the movable arm of the balance can pick up the rider and move it to any desired location on the beam. This eliminates the use of fingers to move the rider. With this type of balance, weight settings of from 1 to 5 milligrams and below 1 milligram are obtained by moving the rider to different locations on the beam. If the rider is accidentally removed from the beam, forceps should be used to replace it on the beam. Weights from 1 to 200 grams are added to the pan using weights provided in the weight set.

Figure 3-5. Rider type analytical balance.

(b) Chain-o-matic analytical balance. This type of balance uses a chain for the weights from 1 gram to 100 milligrams (see figure 3-6). This chain is attached to the beam and to a movable scale in order that the actual weight can be easily read off the scale. Each manufacturer has there own design for this scale, but all scales are similar in nature. In addition, all beams have been modified so that a 0.1 gram rider can be used for 0.1 gram increments from 0 to 1 gram. Thus, the need for adding small milligram weights is eliminated. This reduces the chance of error in adding up the weights on the balance. The rider is shaped like a railway car wheel. The addition of the chain requires the addition of a control knob on the outside of the case so that the chain can be moved by utilizing the movable pick-up arm of the balance in the same manner as that of the rider type balance.

Figure 3-6. Chain-o-matic analytical balance.

(2) Older models availability and characteristics.

(a) Older rider and the chain-o-matic type opposition balances. Although the rider and the chain-o-matic type opposition balances are not available in the Army supply system, they are still used in some laboratories.

(b) Semi-analytical. You may encounter some balances that are loosely referred to as "analytical" balances. However, this term is not fully correct since these balances have a precision of only 0.001 gram (1 milligram). These balances are actually semi-analytical which can be thought of as a sub-type of balance.

(3) Modern balance.

(a) Newer substitution analytical balance. This balance is a single-pan substitution balance with unequal arms. Suspended above the arms are a series of weights that are counterbalanced by a single weight located at the opposite end of the beam. Because the load on either side of the knife-edge is always constant, the sensitivity of the substitution balance does not vary. The material to be weighed is placed inside a tarred container on the weighing pan and weights to nearest 0.1 g are removed from the beam by a dial control lever. Weights of less than 100 mg are read from an optical scale attached to the end of the beam. A light source coupled with appropriate lenses and mirrors project the optical scale (0 to 100 mg) on a screen located on the front of the balance.

(b) Substitution type balance. The more modern substitution balances have their adjustable weights internally hung just above the single pan (see figure 3-7). The transfer of weights, moving of the rider, and manipulating of the chain are therefore completely eliminated. Depending upon the manufacturer, a fixed mass is balanced by a series of fractional weights. Weighing of an object consists of removing weights until the object and the remaining weight equals the fixed mass of the particular type balance being used. Removal of the fractional weights is controlled by calibrated knobs on the case of the balance. The construction, as described earlier, is augmented by an optical system, which visually expands the slight movement of the opposing balance arms. The movement is reflected by mirrors and magnified as it is projected onto a small screen at the front of the instrument. A scale etched in the optical system divides the reflected movement into proportionally spaced lines that are labeled for the corresponding weight. The readout for the adjustable (removable) weights is mechanically displayed in small windows as the knobs controlling the weights are rotated to lift the weights.

Figure 3-7. Substitution type balance.

(c) Top-loader model. The styles of opposition analytical balances (double pan) have sliding, "riding," or hanging "chain" weights that are adjustable for smaller increments of weights. The larger increments of weights are in the form of separate weights that are stored in a special box when they are not being used. Both the opposition and the substitution analytical balances have their arms balanced about a knife edge fulcrum which is usually made of high-quality quartz which maintains a keen edge. These balances work on the same principle as the substitution balance described above. True top-loading analytical balances are available with a capacity of 30 grams and a readability of 0.0001 gram (0.1 milligram). However, most top-loading balances used in the clinical laboratory are semi-analytical with a capacity of 200 grams and a readability of 0.001 gram (1 milligram).

(d) Variation models. Many analytical balances are now completely electronic with automatic weighing and will automatically tare the container weight (weighing dish) and have direct digital output. Many of these electronic balances will interface with automated data processors (such as desk top calculators).

(e) Reagent type models. Analytical balances are employed to weigh small amounts of reagents and are invariably used in weighing standards. Single pan styles usually have an enclosed weighing chamber, while most "top-loaders" have the pan situated openly above the balance housing. Single pan models generally give better results in drafty rooms since the doors to the weighing chamber can be closed.

e. **Torsion Balance**. A torsion balance can usually be found in a pharmacy and has applications in the laboratory when readability is desired intermediate to that of the trip and/or analytical balances. Torsion balances do not have a knife-edge fulcrum. They employ a stationary metal band (or wire) as a fulcrum; the band is attached to the arms at a right angle and is usually centered. As the balance arms move, the band twists. This causes it to exhibit a spring-like force called torsion. When the instrument is balanced, the band is fully relaxed and the torsion is zero. With the exception of the torsion fulcrum, a torsion balance operates much like any two pans, equal arm balance. As the balance oscillates, the movement of the pans is restricted by the torque of the bands. Because of the differing support systems, the torsion balance cannot support as much weight as the knife-edge balance. They can have a readability of 2 mg and provide a more accurate, rapid means of weighing small samples.

(1) Coarse torsion balance. The coarse torsion balances are used for weighing large amounts of substances or for weighing extremely heavy substances such as mercury or lead. These balances usually have a large capacity (up to 5000 grams). The sensitivity of these balances is usually 1 gram. In general, coarse torsion balances have no practical use in the clinical chemistry laboratory.

(2) Pharmaceutical or prescription balance. In the past, these balances were used to some extent because of their semi-analytical precision and availability. Prescription balances have a sensitivity of 0.01 gram (10.0 milligrams) and a capacity of from 100 to 200 grams (see figure 3-8).

Figure 3-8. Pharmaceutical or prescription balance.

f. **Semi-Micro Balance**. These balances are not routinely used in the clinical laboratory since their precision is greater than that which is normally required. The precision of a semi-micro balance is 0.00001 gram (0.01 milligram). The capacity is approximately 100 grams. Both single pan substitution and double pan opposition models are manufactured.

g. **Micro Balance**. The micro balance has practical application in research since its precision is 1.0 microgram ($10^{-6}$ gram). Its capacity is about 1.5 to 30 grams; however, the capacity may vary from model to model.

h. **Ultra-Micro Balance**. This balance is a refinement of the micro balance. The ultra-micro balance has a sensitivity of 0.1 microgram ($10^{-7}$ gram). This type of balance usually has a small weighing capacity (from 1.0 to 3.0 grams depending upon the model). Like the semi-micro and the micro balance, use of the ultra-micro balance is usually reserved for research.

i. **Top-Loading Balances**. Top-loading balances are modified torsion or substitution balances and are much faster and easier to use than other balances. Weighing can be completed in a few seconds, however, precision is not as good as that obtained with analytical balances and ranges from 20 mg to 1 mg, depending on design.

## 3-6. ELECTRONIC BALANCES

a. The electronic balance can equal the precision and accuracy of all types of mechanical balances and are replacing the latter in clinical laboratories. Electronic balances are either top loading or analytical in design and allow weighing to be made in 5 seconds or less. The electronic balance is a single-pan balance that uses an electro magnetic force to counterbalance the load placed on the weighing pan. The pan is attached directly to a coil suspended in the field of a permanent magnet.

b. A current is passed through the coil producing an electromagnetic force that keeps the weighing pan in a constant position. When a load is placed on the pan, a photoelectric cell scanning device attached to the lever arm changes position and transmits a current to the amplifier. This increases the current flow through the coil and restores the pan to its original position. This current is proportional to the weight of the material on the pan and produces a measurable voltage, which is measured by a microprocessor to a measurable display or data output. The balances are usually interfaced with data processing equipment to provide calculations, such as average weight and statistical analysis.

## 3-7. PROPER USE AND CARE OF THE BALANCE

a. The guiding principle in weighing technique is to regard a balance as a delicate, precision instrument, which will function properly only if it is not abused. Ensure that the balance is in a draft free location and not in direct sunlight.

b. This is the sequence in weighing a sample using a single pan balance.

(1) Check that the balance is level by observing the level indicator and make appropriate adjustments to the feet length.

(2) Ensure that the balance is scrupulously clean, especially the weighing pans. Remove any chemicals or dust from the weighing area with a camel's hair or sable brush to avoid corrosion of metal surfaces.

NOTE: Keep the balance and weights as moisture free as possible. It is suggested that a desiccant, such as anhydrous calcium chloride, be placed in the balance to absorb moisture. Never touch the balance pan or weights with the fingers because a grease deposit will be left on the weights, thereby changing the actual weight. Use the tongs or forceps that are provided with the weight sets to handle the weights. Be careful to avoid dropping or scarring weights.

(3) Set the balance to its zero point. If tarring is used, set the readout at zero. For the analytical balance, this setting is made with the sliding windows closed and the beam resting on the knife edge. Release the beam gently. Position so that the knife edges are not in contact with the agate plates. Use the "arrest" (if provided) when adding or removing weights or substances from the pan(s). Also sudden movements can cause the knife edges and the agate plates to damage each other and thus produce inaccuracies in weighing.

**CAUTION**: Never allow the beam to rest on the knife edges while weights or materials are being added or removed.

(4) Lock the beam of the analytical balance. Open the window of the balance case and place the material to be weighed on the weighing pan. Close the window.

(5) Set the beam arrest knob in the intermediate position. Be careful because the knife edge, located at the fulcrum of the beam, is a synthetic sapphire and can be injured by lowering the beam too hard or through excessive vibration. The knife edge and agate plates are the most critical parts of an analytical balance.

NOTE: Check the accuracy of the balance at regular intervals using certified, calibrated weights of analytical quality.

(6) Make gross weight changes until the weight of the material is in the range of the optical scale, with the balance in the beam "arrest" position.

NOTE: On the balances that are encased, the final weighing must be made with the case closed in order to avoid movement of the pans by air currents.

(7) Arrest the beam fully and allow the weighing pan to come to its final point of rest. Perform all manipulations slowly and carefully.

**CAUTION**: Do not overload the balance.

**CAUTION**: Never weigh chemicals directly on the pans as they may corrode the pans, thereby changing the weight of the pan. Use weighing paper, beakers, watch glasses, or other suitable containers for these chemicals. Hygroscopic chemicals (that take up and retain water from the air or any medium are used as drying agents) should never be weighed on filter paper or other absorbent paper because they will soak through and may etch the pan surfaces. Weigh corrosive materials in closed containers.

**CAUTION**: Do not weigh chemicals when they are significantly hotter or colder than room temperature since they tend to produce convection currents that cause inaccuracies.

(8) Record the mass of the material.

(9) Arrest the beam fully and remove the material from the weighing pan.

c. Hygroscopic materials and volatile liquids are difficult to weigh accurately. Solids, which have been dried and placed in a desiccator (drying agent holder), are often hygroscopic and should be weighed in weighing bottles with ground-glass stoppers.

## 3-8. PERFORMANCE

Mechanical and electronic balances, that are maintained and used according to the manufacturer's instructions manual, may not require service more than once a year, depending on the frequency of use. Most mechanical and electronic analytical balances have internal weights that meet the tolerances for class S weights, established by the National Bureau of Standards. The performance and reliability of an analytical balance (often erroneously referred to as "calibration") can be checked if a set of class S weights are available. A 100 g weight should weigh 100 g $\pm$ 0.5 mg. If the class S weights weigh greater or less than the maintenance tolerance values (see table 3-2), the balance should be serviced. Adjustments, other than those described in the balance operating instructions, should be performed by a qualified service technician. Some electronic top-loading and analytical balances have an internal weight built into the balance for calibration.

| NOMINAL MASS | INDIVIDUAL TOLERANCE (MG) | MAINTENANCE TOLERANCE (MG) |
|---|---|---|
| 1, 2, 3, 5, 10, 20, 30, 50 mg | 0.014 | 0.014 |
| 100, 200, 300, 500 mg | 0.025 | 0.05 |
| 1, 2, 3, 5 g | 0.054 | 0.11 |
| 10, 20, 30 g | 0.074 | 0.148 |
| 50 g | 0.12 | 0.22 |
| 100 g | 0.25 | 0.5 |

Table 3-2. National Bureau of Standards tolerance for class S weights.

## Section II. MEASUREMENT OF VOLUME

### 3-9. VOLUMETRIC GLASSWARE

a. **General**. Most experienced laboratory specialists are familiar with the mechanical manipulation of the more commonly used laboratory glassware but are lacking in the knowledge of the accuracies and the specific uses of volumetric glassware (see figure 3-9). You must not only be able to select the type of glassware best suited for the particular measurement but also know how to use the glassware and precautions that should be observed to obtain the best accuracy, with a minimum of wasted time. It should be remembered that for precise work not all laboratory glassware designed either to contain or to deliver complies with the graduation mark on the apparatus. For example, graduated cylinders are used for less exact measurements and the old bell-shaped graduates, used in pharmacies, are not sufficiently accurate for use in the chemical laboratory. Ideally, all volumetric glassware should be calibrated by an experienced technician or purchased with a certification that the volume tolerances meet or exceed the requirements of the U.S. Bureau of Standards. Most of the laboratory glassware utilized in the clinical laboratory today is made of either glass or plastic, both of which may be made of several different types of material. By far the most common type of glassware in measurement of volume is borosilicate glass. This glass is characterized by a high degree of thermal resistance but it is poor technique to store concentrated alkaline solutions that could etch or dissolve the glass and destroy calibration.

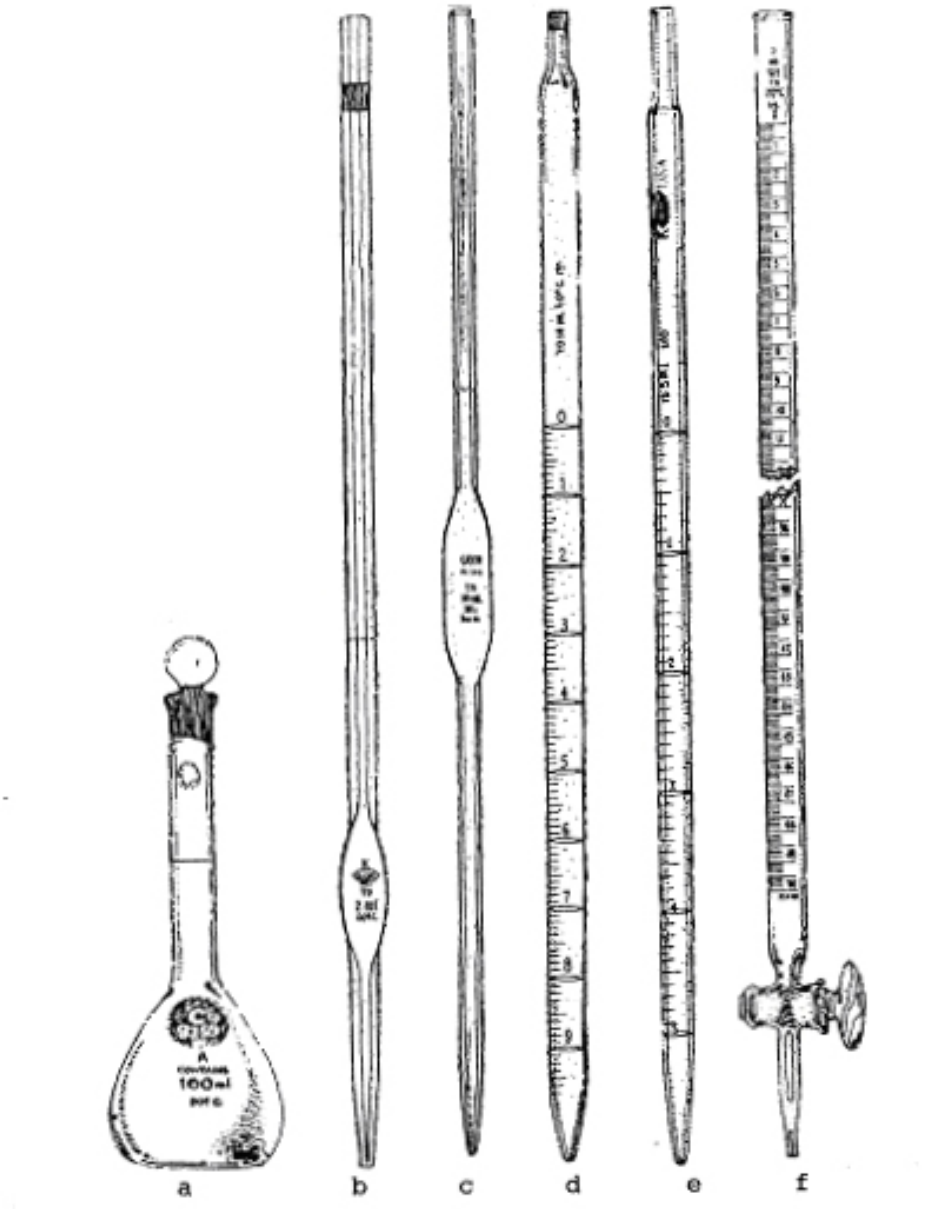



Figure 3-9. Volumetric glassware.

b. **Classification of Volumetric Glassware.** In general, items of volumetric glassware are classified into two main groups which is the basis of whether they contain or deliver a certain quantity of solution when the container is filled with liquid to the measuring mark (see table 3-3).

| TYPE OF PIPET | TRANSFER (Volumetric) | OSTWALD-FOLIN | MICROPIPETS | SEROLOGICAL |
|---|---|---|---|---|
| ACCURACY | Most accurate for accurate solutions<br><br>± 0.5% | Most accurate for viscous solutions<br><br>± 1.0% | Most accurate for small quantities<br><br>± 1.0% | Least accurate<br>Advantages:<br>a. Speed<br>b. Delivery of intermediate volumes<br><br>± 2.0% |
| Common Clinical Use | Aqueous Solutions<br>a. Dilutions of stock standards<br>b. Reconstitution of dehydrated controls<br>c. Preparation and titration of standard acids and bases | Viscous Solutions<br>a. Blood<br>b. Plasma<br>c. Serum<br><br>Aqueous Solutions<br>a. Some reagents<br>b. Some working standards | Small quantities of aqueous and viscous unknowns and standards | REAGENTS |
| Types of Delivery | To Deliver (TD)<br>Drain-out<br>Touch Delivery<br>DO NOT blow out | To contain (TD)<br>Drain-out<br>Blow-out<br>"Frosted Band" | To contain (TC)<br>Rinse-out<br>(3-4 times)<br>NOTE: TD micropipettes do exist | Line to Line delivery<br>Blow-out<br>"Frosted Band" |

Table 3-3. Examples of TD and TC glassware (pipettes).

(1) To deliver (TD) glassware. When a "to deliver" or "TD" container is filled to the mark, it will actually contain slightly more than the designated "to deliver" volume because it is impossible to drain a pipette, buret, or flask completely. The TD containers are calibrated so that small amounts of liquid remain attached to the inner surfaces but that the gaining container will receive the volume shown. This is taken into consideration when the item of glassware was calibrated. Some TD pipettes are designed to be used by "drainage" delivery, others by "blowout" delivery. A TD pipette; which does not have a frosted ring close to the mouthpiece; is allowed to drain without restriction until the fluid level reaches the delivery tip. The tip is then touched to the side of the receiving container and contact maintained until drainage stops. The small amount of fluid remaining in the tip is not blown out. Pipettes, which have a frosted band, are used with "blowout" delivery; the last few drops remaining in the tip are blown out after free drainage is complete.

(2) To contain (TC) glassware. Pipettes of 0.2 ml or less, some graduated cylinders, and most volumetric flasks are usually calibrated to contain a specific volume. This glassware will contain the exact volume of fluid as indicated by the measurement marks. After fluid from a TC pipette, cylinder, or flask has been delivered into a receiving vessel, the pipette, cylinder, or flask is rinsed at least three times with sufficient diluents to make certain that all the fluid adhering to the inner surfaces of the glass is delivered into the receiving vessel. With small pipettes, the ratio of wall surface to volume content is sufficiently large. Differences in the manner of delivery or in the physical properties of the fluid could cause relatively large variations in the percentage of fluid remaining on the inner wall if "TD" delivery were used.

c. **Special Types of Glassware.** Commercial brands are known as Pyrex®, (Corning®), and Kimax®, (Kimble®).

(1) Strength. The Corex®, (Corning®) brand glassware is a special glass strengthened chemically rather than thermally. Corex® is at least six times stronger than borosilicate glass. Corex® is a alkali-resistant glassware which resists clouding and scratching. However, it has only about half the thermal shock resistance of Pyrex® glassware and therefore must be heated and cooled more carefully.

(2) Density. Low actinic glassware is a glass of high thermal resistance with an amber or red color added as an integral part of the glass. The density of the color is adjusted to permit adequate visibility of the contents, yet give maximum protection to light sensitive material, such as bilirubin standards.

(3) Tolerance. Volumetric glassware is classed A, B, and Student Grade. The tolerances for accuracy of the Class A glassware meet or exceed the strict requirements specified by the National Bureau of Standards. All Class A volumetric glassware is the only type acceptable by the College of American Pathologists for use in an approved clinical laboratory.

## 3-10. VOLUMETRIC FLASKS

a. **Description**. Volumetric flasks are essential and are of the most accurate pieces of laboratory glassware for preparation of solutions. Class A specifications are required for such use. Standard supply items range in size from 25 ml to 2000 ml volumes. However, other sizes are available. The typical volumetric flask consists of a large bulbous lower portion with a flat bottom and a long slender neck. A line or an equivalent mark on the neck points out the position at which the meniscus must be located to achieve the stated volume.

b. **Calibration**. Volumetric flasks are essential and are of the most accurate pieces of laboratory glassware for preparation of solutions. Class A specifications are required for such use. The accuracy is usually ± 0.1 percent or better and these flasks must be used when preparing solutions that require a high degree of accuracy. The flask is calibrated "to contain" not deliver, a given volume at 20° C. There are some volumetric flasks which have two lines etched on their necks, one to contain and the other to deliver. This means that a flask contains a specified volume of solution and if the content of a 100-ml flask were poured into another 100-ml flask, it would not contain the original amount. A TD flask will deliver an exact volume of nonviscous liquid. If the temperature is appreciably higher or lower, provision must be made to bring the solution to the calibration temperature before filling the flask to the mark. With elevated temperatures, a more concentrated solution will be obtained. Conversely, when cold solutions are brought to the mark, the solution will be more dilute than the desired concentration. Once the action is complete, the top can be capped with a tight fitting, ground glass stopper. This allows the flask to be inverted for proper mixing.

c. **Proper Care and Use of Volumetric Flasks.**

(1) Heating. Volumetric flasks must not be dried in a hot air oven because the calibration may be altered due to prolonged heating.

(2) Cleaning and drying. Volumetric flasks must be completely dry and clean, especially when they are to be used in the preparation of standard solutions.

(3) Dissolving solute. Several simple procedures are appropriate to dissolve a solute in a given amount of solvent. With the exception of strong acids, the required amount of substance is carefully transferred to the flask and the solvent is added until the flask is about one-half or two-thirds full. This is mixed by agitation until the substance is dissolved. The flask must not be inverted until the volume has been made up to the mark. Otherwise, some of the liquid or substance will adhere to the sides of the neck and stopper and may be lost when the stopper is removed or the liquid adhering to the sides of the flask above the mark will cause excess dilution when the contents are made to the mark. It is advisable to add solvent with a pipette when approaching the mark in order to prevent over-dilution. In any event, the solute must be

transferred into the volumetric flask and dissolved in a small volume of solvent. The solute must be completely dissolved before bringing it to final volume because many solutes have appreciable volume changes when placed in solution. Once dissolved, small portions of solvent are added, with swirling motion, until the meniscus is reached. The volumetric flask is then capped and inverted with a swirling motion several times for 3 to 4 minutes. This should result in uniform mixing.

NOTE: In the case of strong acids, add the water first and then slowly add the acid with gentle agitation. Excessive agitation or combining the two solutions too quickly could result in an exothermic reaction (heat), which may cause a variation in the calibration of the flask.

(4) Transferring solute. After preparing the solution in a volumetric flask, the solution is transferred to a reagent bottle.

**WARNING**

All biological fluids such as cerebrospinal fluid, blood, urine, and sputum, should be considered to contain pathogenic organisms and be treated as an infectious material. Even if the container, pipette, etc., does not contain these items, you are to handle them as if they do.

**WARNING**

Never pipette by mouth, to avoid exposure to HIV, Hepatitis B, or other infectious materials and corrosive or toxic agents. You must wear protective gloves, aprons, and eye protection, and wash hands frequently.

**CAUTION**: Never use volumetric flasks as storage bottles. Alkaline solutions will cause glass stoppers to freeze, making it impossible, other than breaking, to remove the solution.

## 3-11. MANUAL PIPETTES

There are many kinds of pipettes available for use in the clinical laboratory, each intended to serve a specific purpose. In general, pipettes fall into two general classes, volumetric-transfer or graduated-measuring pipettes.

a. **Volumetric or Transfer Pipettes.** The volumetric or transfer pipettes are used primarily when accuracy is very important. This type of pipette has an accuracy of ± 0.06 percent. Most transfer pipettes used in the clinical laboratory are calibrated TD a specific volume by drainage. Such pipettes will have no bands or frosted markings at the mouthpiece. Transfer pipettes are used in clinical chemistry primarily for measuring protein free filtrates, similar non-viscous solutions, and standard solutions. The volumetric pipette is calibrated for one specific volume measurement, either TD or TC. For Class A pipettes, this is clearly indicated on the pipette.

(1) To deliver (TD) pipettes. A TD pipette, which is calibrated for blowout, has an opaque ring near the top. In this case, the amount of liquid remaining in the tip after free delivery has ceased is blown out to allow its addition to the initial volume. To deliver pipettes are also calibrated for the volume delivered, with no attempt to wash out the film which adheres to the inside glass surface. These are named transfer pipettes and are used for measuring protein-free filtrates, similar non-viscous solutions, and standard solutions. The common names given to these pipettes are Ostwald-Folin and serological (long tip and large tip openings).

(a) Ostwald-Folin pipette. The Ostwald-Folin pipette is used to measure blood, serum, plasma, other fluids, or viscous fluids. These pipettes are of a special design in that they are calibrated to deliver one specific volume. Such pipettes have a large oval bulb and a short delivery tip so as to minimize the effects of viscosity in measurements. Accuracy of this type pipette is ± 1.2 percent. These are also TD pipettes but have the additional feature of being "blow out" pipettes. This blow-out feature is designated by a single or double band near the mouth-piece which may either be etched on or painted on; sometimes, depending on the manufacturer, the mouthpiece itself is etched. Any pipette with this etched or banded feature is first allowed to drain and then the last drop or two in the pipette is expelled by blowing gently through the mouthpiece.

(b) Serological pipette. The serological pipette is used mainly for the addition of reagents in specific or intermediate amounts. These pipettes are the least accurate of the routinely used pipettes, having an accuracy of approximately ± 2 percent. All serological pipettes are graduated from the tip to a mark on the upper portion of the barrel. These graduations are machine produced by mass production and account for the high source of error. The main inaccuracy of serological pipettes lies in the tip (bottom) portion, and whenever possible, the use of the tip portion should be avoided. Serological pipettes are also TD blow-out pipettes. Since most procedures in clinical chemistry are designed so that reagents are added in excess, the use of serological pipettes is mainly for the addition of these reagents in a specified volume. Therefore, the addition of a small amount of excess reagent in a test procedure will not influence the results greatly.

(2) To contain (TC) pipettes. A TC pipette is calibrated for total volume of the liquid held in the pipette, and must be washed out completely for delivery of the correct volume. In micro work, the remaining volume that coats the inner wall of a pipette can cause significant error. For this reason, most micropipettes are calibrated TC, the stated volume rather than to deliver it. Most micropipettes, in the range up to 0.5 ml, are calibrated TC.

b. **Graduated Pipettes.** Graduated pipettes are long, cylindrical tubes drawn out to a tip and are calibrated in uniform fractional volume measurements. The **Mohr pipette** type is calibrated between two marks on the stem while the serological type is calibrated to the tip. Accuracy of this type pipette is ± 1.2 percent. All serological pipettes are therefore calibrated for blowout and, accordingly, have the opaque ring at the top for identification. Mohr type pipettes are very similar to serological pipettes. The major difference is that they are not graduated to the tip so they are not blow-out pipettes. The Mohr type of pipette is infrequently used and it is suggested that it not be used. It should be replaced by a serological pipette.

**CAUTION**: Caution must be exercised not to confuse serological and Mohr pipettes because many test procedures can be ruined by the addition of excess reagent with the resulting change in the dilution factor.

c. **Other Pipettes or Pipetting Devices.**

(1) Micropipettes. Volumes are expressed in microliters (µL); the older term lambda is no longer recommended (one lambda = 1 µL = 0.001 ml). Micropipets contain or deliver, ranging from 1 to 1000 microliters (µL). The proper use requires rinsing the pipette with the final solution after delivering the contents into the diluent. The most common type of micropipette used in the laboratory was introduced by the Eppendorf Company. This name has become almost generic for a large number of pipettes that work by the same principle. These are piston operated devices. Disposable and exchangeable tips are placed on the barrel of the pipette. These receive and dispense the liquid. Due to the blowout design of the plunger system, the manufacturer claims to have 99 percent recovery, making it unnecessary to rinse out the tips. The "Eppendorf" type micro and macro pipetting system is produced by a number of manufactures such as Oxford®, Eppendorf®, and MLA® to pipetting systems. Refer to figure 3-10. All of these devices consist of a pipetting "sampler," a device to draw up, hold, and deliver a liquid, and a non-wettable, disposable polypropylene tip. The disposable tip is the pipette and the sampler controls the specified amount of liquid drawn up into the pipette. These pipetting systems are available in several single-range and several multi-range capacities, some with two or three calibrated volumes. These vary from 5 to 1000 µl sizes with a ±1 percent accuracy.

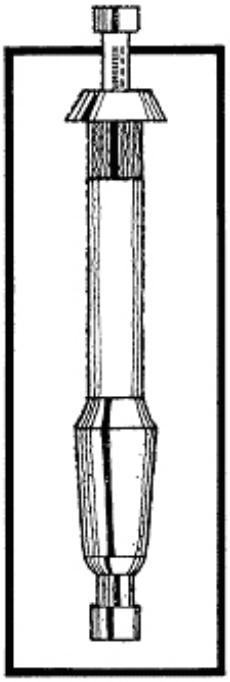


Figure 3-10. Precision pipetting system.

(2) Operation of this system is simple. Simply apply the polypropylene tip to the sampler. Depress the plunger and then immerse the tip (pipette) into the sample solution. Return the plunger to the top release position, allowing the solution to enter the tip. Remove the tip from the sample. Place the tip against the side wall of the receiving vessel and depress the plunger. The non-wettable surface of the tip allows for quantitative transfer of the volume contained in the pipette. Some of these precision pipetting systems are designed to eliminate the requirement of touching the tip (pipette), either in the initial application of the tip or the disposal of the tip. This allows for complete sterile pipetting. This would eliminate such dangers as serum hepatitis, encountered by handling the pipette either by mouth or by hand.

## 3-12. AUTOMATIC AND OTHER KINDS OF PIPETTES AND DISPENSERS

a. Automated dispensers are frequently used by the laboratory to add repeatedly a specific amount of reagent or diluent. Many types of dispensers are available. The Oxford Repipette is a typical example. A long tube leading from the dispenser is placed in the reagent bottle. The dispenser is composed of a plunger, valve system, and dispensing tip. Once the dispensing device is primed with liquid, pressing on the plunger dispenses a pre-selected volume. When the plunger is returned to the original position, the dispensing chamber is refilled. The manufacturer claims accuracy of 1 percent and a reproducibility of 0.1 percent.

b. Repetitive dispensing pipettors (Eppendorf Repeater®, SMI MultiPettor®) are useful devices for that serial dispensing of relatively small volumes of the same liquid. The volume dispensed is determined by pipettor setting and by the size of the disposable syringe-type tip, which also acts as the liquid reservoir.

c. Dilutor-dispensers are often used in automated instruments to prepare a number of samples for analysis. This device pipettes a selected aliquot of sample and diluent into a receiving vessel or instrument. Most of these devices are dual piston type.

## 3-13. BURETS

a. **Description.** The buret is a very accurate device for the dispensing of volume. It is most frequently used in titrations. It consists of a long glass cylinder with graduations corresponding to accurate volumes, a stopcock or other device to restrict the flow of liquid, and a tapered dispensing tip. A buret is filled with liquid and an amount of fluid is allowed to flow into a receiving vessel for waste. The tip is wiped. By opening the stopcock, the liquid is allowed to flow into the receiving vessel, via the buret tip on the side of the vessel, until the meniscus reaches the desired graduation. The reading on the buret is recorded.

b. **Types of Burets.**

(1) Straight burets. This type of buret has only one channel in the stopcock. It is filled by pouring the liquid into the top through a funnel. There are three sizes available in the Army: 10 ml, 25 ml, and 50 ml. This type of buret is used mainly in acid-base titrations for the preparation of standard solutions.

(2) "Automatic" burets. Burets classified as "automatic" have three-way stopcocks to speed up multiple measurements. Whenever possible, the use of "automatic" burets is preferred because a reagent bottle may be attached to the buret in a closed system. This minimizes frequent entries into the reagent bottle and makes the reagent less susceptible to contamination.

(3) Microburets. Microburets, such as the standard 5 ml microburet, are designed to make possible the measurement and delivery of extremely small volumes of solutions and are used primarily in microchemical work. Most of the microburets are of the automatic type and have a three-way stopcock.

c. **Precautions in the Use of Burets.**

(1) Filling burets. Prior to performing a titration, it is essential that all air bubbles caused by filling be removed from the barrel and the tip be completely filled with the solution.

(2) Rate of delivery. The accuracy of the delivered volume is considerably influenced by the rate of delivery since the rate should not exceed the timing specified on the buret. A general rule of thumb is to deliver the fluid in fairly rapid drops (titration) but not in a steady stream because some of the fluid will adhere to the inner surface of the buret.

(3) End point in titrations. When coming to the end of titration, it may be desirable to deliver the last portions of the solution from the buret in fractions of a drop. To do this, a little of the solution is permitted to protrude from the tip of the buret and is detached by touching the tip to the inside of the receiving flask. This is near enough to the solution so that by rotating or tilting the solution, the droplet is mixed with the solution. For convenience, attach an extra tip of rubber tubing to the buret tip. The extra tip is made by drawing out a piece of capillary tubing. The finer tips deliver smaller drops and the extra length enables one to deposit the droplet where it can be easily mixed with the solution in the receiving flask.

(4) Reading of the meniscus. The reading is usually taken at the bottom of the meniscus because it is more sharply defined than the top of the meniscus. Exceptions must be made when solutions (e.g., potassium permanganate and iodine solutions) are so dark that the bottom of the meniscus cannot be seen. In these cases, the position at the top of the meniscus is read. It is essential that all readings are taken at eye level with the meniscus; otherwise, errors from parallax will occur. A piece of white paper with a darkened area is used as background against which the bottom of the meniscus can be seen.

**CAUTION**: Do not add extra tip during titration.

(5) Stopcocks. The most troublesome spot on the buret is the stopcock. It must be greased to prevent "freezing" and leakage. The stopcock and the area in the buret where it is fitted must be completely dry before greasing. The grease is applied in a thin layer on both sides of the capillary openings. The stopcock is then inserted and turned several times to ensure complete sealing.

NOTE: Insufficient lubricant, incomplete drying, and ill-fitting stopcocks will cause leakage. Excessive lubricant will cause the capillary channels to become plugged.

**CAUTION**: Alkaline solution should not be left in a buret for extended periods. The stopcock is likely to "freeze" due to dissolving of the stopcock. Also, dilute alkaline standard solutions of 0.1 N or less may dissolve enough silicate from the glass to change the normality.

(6) Cleanliness. Unless the buret is permanently attached to a reagent bottle, it is rinsed with tap water and distilled water (deionized water) immediately after use, and inverted with the stopcock open to dry. If it is necessary to use the buret again before it is dry, it can be used, provided that the buret is thoroughly rinsed with the solution to be used.

## 3-14. FACTORS IN THE SELECTION AND USE OF PIPETTES

a. **Size**. In addition to selecting the proper type of pipette, the size of the pipette must also be taken into consideration. This is applicable only in serological pipettes, since volumetric and Ostwald-Folin pipettes are designed to deliver only a specified volume. When using serological pipettes, the smallest pipette that will hold the desired volume should be selected. For example, to measure 3.2 ml of reagent, a 5 ml pipette should be used rather than a 10 ml pipette. It is also more accurate to deliver the fluid from the 0 mark to the 3.2 ml mark rather than from the 1.8 ml mark to the tip. The reason for this action is that the greatest inaccuracy of the serological pipette lies in the end portion (tip).

b. **Accuracy**. With the exception of micropipettes, the accuracy of the pipette increases as its holding capacity increases. Whenever sufficient volumes of reagents and specimens are available, a larger rather than smaller pipette will give more accurate measurements. This fact is not particularly adaptable to actual test procedures, but should be considered in the preparation of reagents or in making initial dilutions from the specimens. Supply economy must also be taken into account since it is impractical to use large volumes of expensive reagents to obtain a slight increase in accuracy.

c. **Temperature Effect**. Practically all pipettes manufactured in the United States are calibrated either TC or TD, the specified volume of solution at 20° C. No correction factor is needed if the liquid to be pipetted is at room temperature. However, if the liquid is hot or has just been taken out of the refrigerator, it must be allowed to reach room temperature before it is pipetted.

d. **Speed.** In certain clinical laboratory procedures, the choice of pipette is influenced by the necessary time required or allotted. Additional speed can be gained when using the serological pipette that in turn may increase the accuracy due to better timing. If you had to run 20 of the same determination and used a volumetric pipette to add 2 ml of reagent to each test, the timing would be considerable. But by using a serological pipette, the 2 ml aliquots can be delivered four times faster. The more productive use of time outweighs the slightly greater error introduced by the serological pipette.

e. **Poisonous or Corrosive Reagents**. Quite frequently, poisonous or corrosive reagents must be pipetted in the clinical laboratory. The label will normally specify which reagents are poisonous or corrosive.

**WARNING**

Never pipette by mouth to avoid exposure to HIV, Hepatitis B, or other infectious materials and corrosive or toxic agents. You must wear protective gloves, aprons, and eye protection, and wash hands frequently.

(1) One type of pipetting device used for these types of reagents is the Propipet®, which consists of a rubber bulb with three separate air valves that remain closed unless squeezed (see figure 3-11). The top valve (valve A) allows air to enter the bulb. This valve is also used to evacuate the bulb. By opening the top valve and squeezing the bulb, a vacuum is formed. The lower valve (valve S) opens the passage from the bulb to the attached pipette. This creates the suction required to draw the liquid into the pipette. Liquid, in the pipette, can be adjusted slowly by controlling pressure on the lower valve while maintaining pressure or vacuum in the bulb. A side valve (valve E) allows direct flow of air into the pipette breaking the vacuum. This allows free drainage of liquid in the pipette. To deliver the last drop, maintain pressure on the side valve. At the same time, cover the side valve opening with your finger tip and squeeze the small bulb.

(2) Since liquid entering the lower valve will cause a Propipet® to leak and contaminate other samples, it is good practice to keep one hand on the side valve while filling a pipette. If the liquid rises near the lower valve, a squeeze on the side valve will stop it.

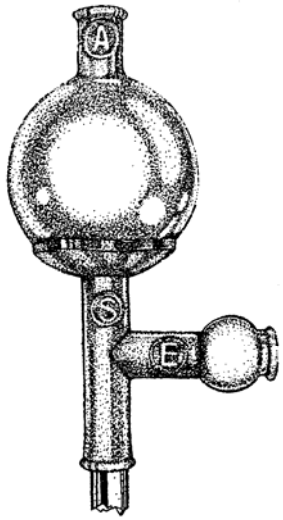



Figure 3-11. Propipet®.

f. **General Pipetting Technique.** The following is a discussion of the general technique for using pipettes as described above. First and foremost, check pipettes for cleanliness, dryness, and glass flaws like chips and cracks. Attach your pipetting device carefully and draw the fluid to be transferred to a point slightly above the etched line. Maintaining the vertical position, wipe off the outside of the pipette with a gauze or lab tissue. Bring the liquid down to the mark-volume to dispense. Allow the liquid to fall slowly and stop when the bottom of the meniscus reaches the proper mark (see figure 3-12). If the level falls below the desired mark, repeat the operation. Insert the pipette into the container and allow it to drain. If the pipette has a frosted band or double ring, blow out the last drop after the contained fluid has come to a rest.

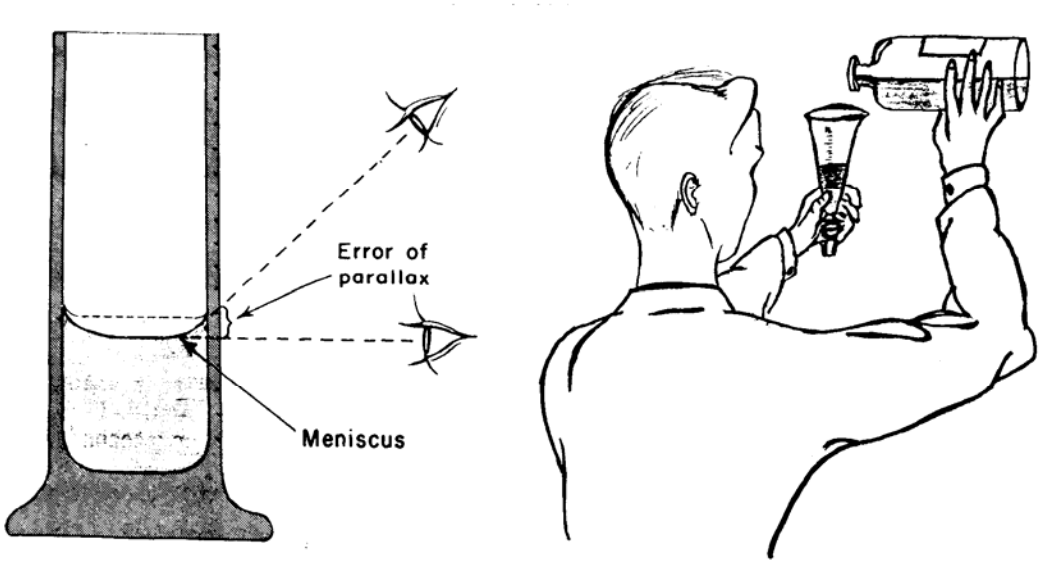



Figure 3-12. How to read the meniscus at eye level.

(1) Using thumb and forefinger press on valve "A" and squeeze bulb with other fingers to produce a vacuum for aspiration.

(2) Insert pipette into liquid. Press on valve "S." Suction draws liquid to desired level.

(3) Press on valve "E" to expel liquid.

(4) Deliver the last drop, by maintaining pressure on valve "E." Cover the "E" inlet with your middle finger and squeeze the small bulb.

NOTE: Special care must be taken in handling transfer pipettes, as chipped tips can influence the actual volume delivered.

**WARNING**

All biological fluids such as cerebrospinal fluid, urine, blood, and sputum, should be considered to contain pathogenic organisms, and be treated as infectious material. Even if the container or pipette does not contain these items, you are to handle them as if they do.

**WARNING**

Never pipette by mouth to avoid exposure to HIV, Hepatitis B, or other infectious materials, and corrosive or toxic agents. You must wear protective gloves, aprons, and eye protection, and wash hands frequently.

## 3-15. CLEANING OF GLASSWARE

a. Glassware for general laboratory use should be rinsed immediately and placed in a weak and hot detergent solution. Later, the glassware must be rinsed thoroughly in tap water and then in deionized (distilled) water. After the glassware is air dried, it must be free of bubbles, water marks, or other potential sources of impurities.

b. The surface of thoroughly cleaned glassware will become uniformly wet, with no adhering water droplets, traces of oil, etc. Special treatment is required in cases of stubborn grease and other organic residues. Let the glassware stand overnight in a sulfuric-dichromate mixture, prepared by pouring 1000 ml of concentrated sulfuric acid into 35 ml of saturated sodium dichromate. Rinse glassware thoroughly after removal from the mixture.

**CAUTION**: AVOID contact with skin or clothing. When glassware must be treated with a special acid solution before being cleaned, use protective covering for your clothes, hands, and eyes. Be aware that all of the liquid may not have dried so use caution when handling the glassware and other instruments.

**WARNING**

All biological fluids such as cerebrospinal fluid, urine, blood, and sputum, should be considered to contain pathogenic organisms and be treated as infectious material. To prevent the release of aerosols, do not open centrifuges until they come to a complete stop.

**WARNING**

Never pipette by mouth to avoid exposure to HIV, Hepatitis B, or other infectious materials and corrosive or toxic agents. You must wear protective gloves, aprons, and eye protection, and wash hands frequently.

**Continue with Exercises**

**EXERCISES, LESSON 3**

**INSTRUCTIONS:** Answer the following exercises by marking the lettered response that best answers the question, by completing the incomplete statement, or by writing the answer in the space provided at the end of the question.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Which statement is correct concerning the use of a balance for modern clinical laboratories?

   a. Fecal fats may be measured by external gravimetric analysis.

   b. Volumetric equipment is calibrated by measurement of gravimetric mass.

   c. Reagents, standards, and controls need to be made from scratch or may be reconstituted.

   d. Measurement of mass is fundamental to every analysis and therefore, the use of some sort of balance is inevitable.

2. In the equation WEIGHT = MASS x GRAVITY, what does mass represent?

   a. A measurement of an object's resistance to a change in motion.

   b. A ping-pong ball moving at 1 cm/sec can be easily stopped by a brick wall.

   c. A bowling ball moves at a different speed than the ping pong ball.

   d. Two substances of different weight and subject to the same gravitational force.

3. If two substances of equal mass and subject to the same gravitational force have equal weight, then:

   a. Changing the location of the balance to places where the gravitational force differs will not affect the function of the balance.

   b. Changing the location of the balance to places where the gravitational force differs will not affect the function of the balance, because the weight of the substance is sometimes relative to the weight of the calibrated mass.

   c. Changing the location of the balance to places where the gravitational force differs will not affect the function of the balance, because the weight of the substance is always relative to the weight of the calibrated mass.

   d. Changing the location of the balance to places where the gravitational force differs will affect the function of the balance.

4. All balances require a _____ free location.

   a. Static.

   b. Clear.

   c. Vibration.

   d. Clean.

5. Which type of substances should never be placed in direct contact with the weighing pans or be permitted to remain on or around the immediate area of the weighing pans?

   a. Chemicals.

   b. Loose crystals.

   c. a and b.

   d. Liquids with corrosive vapors.

   e. a, b, and d.

6. Which type of balance has a capacity of 100 g and a repeatability of 0.01 mg?

   a. Torsion balance.

   b. Semi-micro balance.

   c. Analytical balance.

   d. Trip balance.

7. A single pan balance is suspended from one position on one balance arm along with all of the **adjustable weights**. A stationary counterweight is attached conversely to the first arm and on the other side of the fulcrum. This principle of weighing is said to be:

   a. Substitution.

   b. Direct opposition.

   c. Analytical.

   d. Trip balance.

8. Which type of balance consists of two pans of equal mass suspended from the ends of a beam that is supported at its center of gravity by a knife-edge fulcrum?

   a. Torsion balance.

   b. Top loading balance.

   c. Trip balance.

   d. Ultra micro balance.

9. Which type of balance is a modified torsion or substitution balance and is much faster and easier to use than other balances?

    a. Electronic.

    b. Top-loading.

    c. Analytical.

    d. None of the above.

10. An analytical balance is:

    a. Used for weighing applications which do not require great precision like large amounts of chemical substances or where precision does not exceed 0.1 gram.

    b. Applying practical application in research to a precision of 1 mg and having the capacity of about 1.5 to 30 g.

    c. Widely used in the laboratories since it has greater precision for weighing certain substances and has a varying weighing capacity of from 100 to 200 g.

    d. Weighing masses quickly when a weight to the nearest 0.1 g is satisfactory, as in the preparation of reagents such as strong bases and salts.

11. Which statement is correct concerning the more modern analytical substitution type balance?

    a. The movable arm of the balance can pick up the rider, move it on the beam, and eliminate the use of fingers.

    b. Adjustable weights are externally hung above the single pan so transferring them and the rider and manipulating the chain are completely eliminated.

    c. All beams have been modified so that a 0.1 gram rider can be used for 0.1 gram increments from 0 to 1 gram.

    d. Adjustable weights are internally hung just above the single pan and transferring the weights, moving the rider, and manipulating the chain are completely eliminated.

12. The electronic balance:

    a. Can equal the precision and accuracy of all types of mechanical balances.

    b. Allows weighing to be made in 5 seconds or less.

    c. a and b.

    d. Has a current that is passed through the coil, producing an electromagnetic force that keeps the weighing pan in a constant position.

    e. Can interface with data processing equipment to provide calculations such as average weight and statistical analysis.

    f. a, b, d, and e.

13. Select the statement which describes the proper care of the balance.

    a. The accuracy of the balance should be checked at regular intervals.

    b. The balance can be stored in direct sunlight to keep moisture from building up on the weighing pans.

    c. Noncorrosive chemicals can be weighed directly on the weighing pans to increase accuracy.

    d. The weighing pans should be cleaned monthly with a mild abrasive to remove corrosion on metal surfaces.

14. Which statement describes the proper care and use of the balance?

    a. The beam of the balance should be allowed to rest on the knife edges while weights and/or materials are being added or removed.

    b. Chemicals, that are hot, can be weighed without causing inaccuracy in the weight.

    c. The weights of the balance can be handled with the fingers to prevent scratching.

    d. Remove any chemicals or dust from the weighing areas using a camel's hair or sable brush.

15. What should be placed between the pan and the chemicals when weighing corrosive chemicals?

    a. Watch glasses.

    b. Weighing paper.

    c. Beakers.

    d. Other suitable containers.

    e. All of the above.

16. Hygroscopic materials and volatile liquids are difficult to weigh accurately. Therefore, after drying, they should be:

    a. Placed in a desiccator and weighed in weighing bottles with ground-glass stoppers.

    b. Placed in a glass bottle with a metal stopper and weighed.

    c. Weighed in the normal manner.

    d. Weighed in mass while the beam is fully arrested.

17. To maintain accuracy and performance, mechanical and electronic balances are:

    a. To be serviced according to local standards.

    b. Maintained and used according to the manufacturer's instructions manual.

    c. Maintained according to the commander's wishes.

18. How can the performance and reliability of analytical balances be checked with a set of class S weights?

    a. If the class S weights weigh the same as the maintenance tolerance values, the balance should be serviced.

    b. If the class S weights weigh greater or less than the maintenance tolerance values, the balance should be serviced.

    c. If the maintenance tolerance values remain constant, then balance should be serviced.

    d. All of the above.

19. Who should make or perform adjustments to mechanical and electronic balances, other than those described in the balance operating instructions?

    a. The NCO.

    b. The civil service worker.

    c. A qualified service technician.

    d. An MOS 92B40 NCO.

20. In checking the performance of a mechanical balance, if the nominal mass is 50 g and the individual tolerance is 0.12 mg, what should the maintenance tolerance be for calibration to be accurate?

    a. 0.014 mg.

    b. 0.148 mg.

    c. 0.184 mg.

    d. 0.22 mg.

21. An experienced laboratory specialist, familiar with the mechanical manipulation and knowledgeable in the accuracy of volumetric glassware, would:

    a. Select the best type of glassware suited for the particular measurement and know how to use the glassware.

    b. Follow the necessary precautions to obtain the best accuracy with a minimum of wasted time.

    c. a and b.

    d. None of the above.

22. Which statement is correct?

    a. Graduated cylinders are used for less exact measurements and the old bell-shaped graduates are not sufficiently accurate for chemical laboratory use.

    b. Sometimes, some volumetric glassware should be calibrated by an experienced technician.

    c. Pipettes are used for less exact measurements and the old bell-shaped graduates are not sufficiently accurate for chemical laboratory use.

    d. All of the above.

23. What is the most common clinical laboratory glassware made for today that measures volume?

    a. Sand glass.

    b. Plastic.

    c. Borosilicate glass.

    d. Stainless steel.

24. Borosilicate glass:

    a. Has a high degree of thermal resistance.

    b. Is a good place to store concentrated alkaline solutions.

    c. Is a good container because it allows alkaline solutions to etch or dissolve the glass.

    d. Is really a plastic.

25. Which definition of TD glassware is correct?

    a. A TD container will deliver all fluid within 0.9 ml of volume shown on the container.

    b. A TD container has an error factor of only 0.2 measured.

    c. A TD container will deliver the volume shown on the container.

    d. A TD container will contain the volume marked on the container, but only by blowing it out.

26. Select the correct definition of TC glassware.

    a. A container that will deliver the volume shown on the container.

    b. A container that will deliver fluid within 0.9 ml of volume shown on the container.

    c. A container that has an error factor of only 0.2 measured.

    d. A container that contains the exact volume of fluid as indicated on the container.

27. When using a volumetric flask at room temperature but the solution has an elevated temperature, what will happen to the final volume of solution because the flask is calibrated at 20° C ?

    a. It will become more dilute.

    b. The solution will become more concentrated.

    c. a and b.

    d. The solution remains the same.

28. When using a volumetric flask to dissolve a solute in a given amount of solvent, what sequenced procedures are to be followed?

    (1) Except for strong acids, the required amount of substance is carefully transferred to the flask and the solvent is added until the flask is about one-half or two-thirds full.

    (2) Add the solvent with a pipette when approaching the mark in order to prevent over-dilution.

    (3) Mix by agitation until the substance is dissolved.

    (4) Put a stopper in the volumetric flask, invert with a swirling motion several times for 3 to 4 minutes.

    (5) Once dissolved, small portions of solvent are added, with a swirling motion, until the meniscus is reached.

    a. 5, 4, 2, 3, 1.

    b. 2, 5, 3, 4, 1.

    c. 1, 4, 2, 3, 5.

    d. 1, 3, 2, 5, 4.

29. From the following group of statements below, select the statement associated with the use of volumetric flasks.

    a. They should be rinsed with tap water prior to use in order to coat the inner lining with an ion-free covering.

    b. Strong alkaline solutions can be stored in volumetric flasks.

    c. Volumetric flasks should be heated in hot air ovens prior to use.

    d. When using volumetric flasks, diluting strong acids should be accomplished by adding the water first, then slowly adding the acid to the water.

30. Why must you NEVER use volumetric flasks as storage bottles?

    a. Alkaline solutions will cause glass stoppers to freeze, making it impossible, other than breaking, to remove the solution.

    b. Alkaline solutions will cause glass stoppers to crumble and allow the solute to ooze out.

    c. Acidic solutions will cause glass stoppers to freeze, making it impossible, other than breaking, to remove the solution.

    d. Acidic solutions will cause glass stoppers to crumble and allow the solute to ooze out.

31. Pipettes are intended to serve a specific purpose and, in general, fall into two general classes which are:

    a. Volumetric and transfer.

    b. Transfer or measuring.

    c. Volumetric and graduated.

    d. Graduated or measuring.

32. Which pipette is NOT calibrated for blowout?

    a. Otswald-Folin.

    b. Serological.

    c. Mohr.

    d. TD pipette.

33. What is an automatic pipette dispenser composed, why is it frequently used in the laboratory, and what is its accuracy?

    a. The dispenser is composed of a plunger, valve system, and dispensing tip; it is used to add repeatedly a specific amount of reagent or diluent: its accuracy is 0.1%.

    b. The dispenser is composed of a plunger, valve system, and dispensing tip; it is used to add repeatedly a specific amount of reagent or diluent; its accuracy is supposedly 1%.

    c. The dispenser is composed of a plunger, valve system and stopcock; it is used for dispensing infrequent amounts of diluents; its accuracy is 0.001%.

    d. The dispenser is composed of a plunger and dispensing tip; it is used to prime liquids; its accuracy is 0.111 eye level with the meniscus.

34. You wish to measure 0.01 ml of a particular solution. From the types of glassware listed below, select the type of glassware that would be most appropriate to use.

    a. An appropriate sized micropipette.

    b. 0.1 ml Ostwald-Folin.

    c. 1 ml transfer pipette.

    d. 1 ml volumetric flask.

35. You wish to measure 2.0 ml of blood. From the types of glassware listed below, select the type of glassware that would be most appropriate to use.

    a. 2.0 ml volumetric flask.

    b. 2.0 ml Ostwald-Folin pipette.

    c. 0.2 ml micropipette.

    d. 5.0 ml serological pipette.

36. You wish to measure 10 ml of a non-viscous, protein-free filtrate. From the types of glassware listed below, select the type of glassware that would be most appropriate to use.

    a. 10 ml Ostwald-Folin.

    b. 10 ml transfer pipette.

    c. 15 ml buret.

    d. 10 ml volumetric flask.

37. What happens to liquid when the buret is full and the stopcock is opened?

    a. The liquid flows into the receiving vessel, via the buret tip on the side of the vessel, until the meniscus reaches the desired graduation.

    b. The liquid flows into the plastic tube via the buret tip on the side of the vessel.

    c. It vaporizes and is expelled via the buret tip on the side of the vessel.

    d. It becomes thicker and remains there until the buret is heated.

38. Select the statement which best describes an important factor in the selection of a pipette.

    a. Concentrated acids should be pipetted by mouth in order to be as accurate as possible.

    b. Inorganic solutions should be pipetted at a minimum of $37^{o}$ C for accuracy.

    c. A pipetting device should not be used to pipette organic acids.

    d. The smallest pipette, which will hold the desired volume, should be selected.

39. Listed below are some steps that should be followed in cleaning glassware. As they are listed, the steps are not in order. Select from the answers, the response that best lists the proper sequence in cleaning steps.

    (1) Immerse glassware in dilute detergent solution of hot water.

    (2) Rinse the fluid being measured from the container.

    (3) Rinse the glassware in tap water.

    (4) Rinse the glassware in deionized (distilled) water (when indicated).

    (5) Air dry the glassware.

    (6) Check the glassware for bubbles, water marks, and other potential sources of impurities.

    a. 1, 2, 3, 4, 6, and 5.

    b. 2, 1, 3, 4, 6, and 5.

    c. 5, 2, 3, 1, 4, and 6.

    d. 2, 1, 6, 4, 3, and 5.

40. What type of special treatment is required of glassware when stubborn residuals remain?

    a. Thoroughly clean the glass surface.

    b. Let the glassware stand overnight in a sulfuric-dichromate mixture and then thoroughly rinse it after removing the mixture.

    c. Rinse glassware thoroughly.

    d. Press on the "E" valve to expel the residuals.

**Check Your Answers on Next Page**

**SOLUTIONS TO EXERCISES, LESSON 3**

1. d (para 3-2a)
2. a (para 3-2b)
3. c (para 3-2b)
4. c (para 3-2c)
5. e (para 3-2c)
6. b (para 3-5f, Table 3-1)
7. a (para 3-3a)
8. c (para 3-5a)
9. b. (para 3-5i)
10. c (para 3-5d)
11. d (para 3-5d(3)(b))
12. f (para 3-6)
13. a (para 3-7b(5) NOTE)
14. d (para 3-7b(2))
15. e (para 3-7b(7) 2nd **CAUTION**)
16. a (para 3-7c)
17. b (para 3-8)
18. b (para 3-8)
19. c (para 3-8)
20. d (table 3-2)
21. c (para 3-9a)
22. a (para 3-9a)

23. c (para 3-9a)

24. a (para 3-9a)

25. c (paras 3-9b(1), 3-11a(1))

26. d (para 3-9b(2))

27. b (para 3-10b)

28. d (para 3-10c(3))

29. d (para 3-10c(3) NOTE)

30. a (para 3-10c(4) **CAUTION**)

31. c (para 3-11)

32. c (para 3-11b)

33. a (para 3-12a)

34. a (para 3-13a)

35. b (para 3-11a(1)(a))

36. b (para 3-11a)

37. a (para 3-13c(5))

38. d (para 3-14a)

39. b (para 3-15a)

40. b (para 3-15b)

**End of Lesson 3**

## LESSON ASSIGNMENT

**LESSON 4** Introduction to Quality Control.

**TEXT ASSIGNMENT** Paragraphs 4-1 through 4-10.

**LESSON OBJECTIVES** After completing this lesson, you should be able to:

4-1. Select the statement that best defines and uses the following terms appropriately: quality control system, accuracy, precision, mean, standard deviation, coefficient of variation, shift, trend, and out of control.

4-2. Select the statement that best describes the major application for both internal and external quality control systems and the type of specimens used.

4-3. Select the statement that best describes the possible sources of error in quality control procedures.

4-4. Calculate the mean, standard deviation, and coefficient of variation.

4-5. Select the statement that best describes the data to construct a Levey-Jennings quality control chart and correctly interprets the results.

4-6. Select the statement that best describes the interpretation of data from the Westgard multi-rule control chart.

**SUGGESTION** After studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

# LESSON 4

# INTRODUCTION TO QUALITY CONTROL

## Section I. QUALITY CONTROL SYSTEM

### 4-1. QUALITY CONTROL SYSTEM

a. Quality control in laboratory medicine has been defined as the study of those errors that are the responsibility of the laboratory and the procedures used to recognize and minimize them. An alternative term, "quality assurance," has been used to represent the techniques available to ensure with a specified degree of confidence that the results reported by the laboratory are correct. In order to have such confidence, there must be both "accuracy control" and "precision control" performed in the laboratory.

b. Quality control is a system by which we as laboratory personnel determine the reproducibility of a laboratory procedure. We mean the sum of our efforts to achieve the highest degree of excellence, so that both the patient and physician obtain correct information in the shortest possible time. Quality control programs frequently use the terms **standard** and **control**.

(1) A standard is a substance of known composition, the value of which is established by an analytical procedure different from the one used in the clinical laboratory (reference method). If the laboratory is able to duplicate the standard value, we can accept the procedure as accurate. **Accuracy** is defined as the closeness of a test result to the true value. Accuracy implies freedom from error.

(2) A control resembles the unknown specimen (e.g., serum or urine) and contains various substances of known concentrations that are assayed by typical clinical laboratory methods. (There are also controls which are not assayed but are used to verify reproducibility).

NOTE: The Federal Food and Drug Administration (FDA) requires that you buy only controlled materials that are free of HIV, hepatitis B virus, other infectious materials, and corrosive or toxic agents. Noncommercial frozen pools should not be used if there is any evidence of the presence of these infectious agents.

**WARNING**

All biological fluids such as cerebrospinal fluid, urine, blood, and sputum, should be considered to contain pathogenic organisms and be treated as infectious material. Even if the container of a controlled or patient specimen does not contain any of these items, you are to handle them as if they do.

**WARNING**

Never pipette by mouth to avoid exposure to HIV, Hepatitis B, or other infectious materials and corrosive or toxic agents. You must wear protective gloves, aprons, and eye protection, and wash hands frequently.

(2) Controls should be assayed by the laboratory along with the unknown samples. The results are used for a calculation of the mean and standard deviation of a given test. Control specimens are used to measure **precision**, which is the closeness of a test result to each other and implies freedom of variation. Control specimens may vary in composition and are NOT used as standards. In order to gain a grasp of quality control, you must be familiar with the use of the terms **accuracy** and **precision** (see figure 4-1). Accuracy refers to how close the value of a determination is to the actual value of a specimen. For example, if several tests all indicate a urea nitrogen value of 20.5 mg percent when its actual value is 27 mg percent, we have poor accuracy. Precision refers to the internal consistency of our value. For example, if we perform a urea nitrogen test and obtain the values of 27.0, 27.2, and 27.1 mg percent, we still have excellent precision, but very poor accuracy if the result should be 20.5 mg percent.

c. Major applications currently in use include **internal** and **external** quality control systems. Internal quality control uses samples of known analytic content. Internal quality control procedures provide predominately precision statistics. Internal quality control should be thought of as a system for assuring the quality of total laboratory performance. External control (proficiency testing) provides periodic unknown samples to thousands of laboratories. Compilation of the data from these programs will provide periodic benchmark accuracy or bias estimates to individual laboratories; both types of programs use similar control materials and statistical approaches.

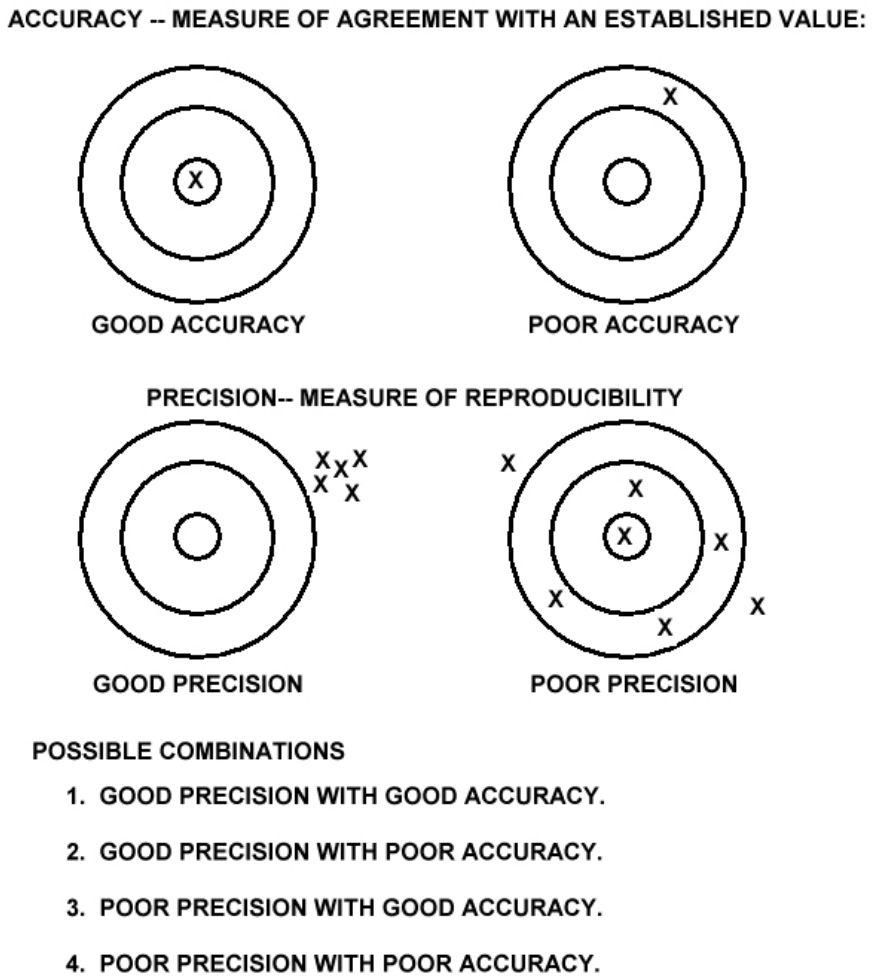



Figure 4-1. Accuracy and precision.

## 4-2. COMMON ERRORS

a. Quality control has as one of its objectives to eliminate errors. Errors frequently result from failing to observe basic precautions and laboratory rules. Some of the more frequent sources of error include the following:

(1) Improper identification of patients and/or specimens.

(2) Failure to adhere to laboratory procedures.

(3) Incorrect, inadequate, or contaminated specimens.

(4) Lack of basic mathematics skills.

(5) Improperly labeled or stored reagents (this includes incorrect type of container and storage temperature).

(6) Transcription errors in laboratory reporting or entering correct laboratory results for the wrong patient.

b. Routine quality assurance is the use of standards and control specimens, proficiency testing, and other aspects of process control. These will not avoid errors in analysis due to something in the specimen (so called matrix factor) nor will such a program prevent errors of interpretation of some unexpected physiologic or genetic factor, a drug, dietary component, or environmental factor.

c. Surprisingly, few laboratory workers pay much attention to matrix errors and even fewer clinicians are aware of their existence. A careful system of specimen collection and handling will avoid most of the problems that are not inherent in the specimen itself.

d. Every clinical laboratory should have a system to detect matrix errors. At a minimum, a list of the most frequently used drugs and the laboratory procedures with which they might interfere should be posted and read.

## Section II. QUALITY CONTROL IN CLINICAL CHEMISTRY

### 4-3. QUALITY CONTROL IN CLINICAL CHEMISTRY

A variety of statistical control techniques have been used in clinical chemistry laboratories, most often on a manual basis. Tabular records, with appropriate calculations, can be used to implement the techniques but graphical displays are often easier to interpret. Tabular data does not readily reveal subtle changes that may be occurring with an analytical method. Therefore, control charts have been accepted as a more effective way to implement most control techniques. The Levey-Jennings chart has been the most widely used technique.

## 4-4. MEAN (ARITHMETIC) AVERAGE

a. The mean (x) of a set of data is used as the point from which we measure deviations. The mean is calculated by finding the sum of the data and dividing by the number of individual readings. For example, suppose we have the measurements 42.0, 42.25, 41.75, and 39 percent transmission for the same specimen. In such a case, we reject the 39 percent reading because of its deviation from the average established by the other readings. The mean is calculated by finding the sum of the data and dividing by the number of individual readings. The formula is:

$$\overline{X}\,(\text{mean}) = \frac{X_1 + X_2 + X_3 + X_n}{n}$$

NOTE: Reject 39%. Divide by "n" (the number of individual readings) to obtain the mean.

$$\overline{X}\ (mean) = \frac{42.0 + 42.25 + 41.75}{3}$$

$$\overline{X} = 42$$

b. If the mean (and the deviations to be measured from it) is to have any significance, a large amount of data must be obtained, preferably 20 data points or more. In cases where fewer than 20 observations are collected, we have no way of knowing if our results are misleading due to unusually large random variations in measurement. With large sets of data (observations), this possibility becomes more remote.

## 4-5. STANDARD DEVIATION

a. The determination of precision of a method and of the significance of differences between determinations is carried out by determining the mean and standard deviation. This is a mathematics computation of the different values obtained in a test series and the difference from the average value.

b. The mathematical formula for **standard deviation** (SD) of a number of values (N) is:

$$SD = \sqrt{\frac{\sum(x_i - \overline{x})^2}{N-1}} \qquad SD = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 1}}$$

(1) Expressed in words, this means that one calculates the mean value of the determinations ($\bar{x}$), finds the difference between the separate values of ($x_i$), squares these differences $(\bar{x} - x_i)^2$, and finds the sum of these squares ($\Sigma$ indicates the summation). This sum is then divided by one less than the number of values (N - 1) and the square root of the quotient is extracted. One standard deviation should be rounded to one more decimal place than the data set. Two and three standard deviation ranges should be rounded to the same accuracy as the data set.

(2) For example: Determine the standard deviation of the following numbers: 3, 6, 9, 12, and 15.

(a) Step 1: Prepare the table.

| Number | Mean | Mean - Number | (Mean Number)$^2$ |
|---|---|---|---|
| 3 | 9 | 6 | 36 |
| 6 | 9 | 3 | 9 |
| 9 | 9 | 0 | 0 |
| 12 | 9 | -3 | 9 |
| 15 | 9 | -6<br>0 (quick check. Sum must equal 0) | 36<br>90 = Sum of squared differences from mean |

(b) Step 2: Use the formula.

$$SD = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 1}}$$

$$SD = \sqrt{\frac{90}{5-1}} = \sqrt{\frac{90}{4}} = \sqrt{22.5} = 4.74$$

(c) Step 3: One standard deviation should be rounded to one more decimal place than the data set.

c. The standard deviation is the range of dispersion (distribution) of values about their mean. The standard deviation (SD) is used as the measure of reproducibility or repeatability; it serves to establish confidence limits by which one can predict. When the standard deviation of a number of determinations (20 or more) is calculated, approximately 68 percent of all values will fall within 1 $\pm$ SD from the mean, 95 percent within $\pm$ 2 SD, and 99.7 percent within $\pm$ 3 SD. Please note that the greater the standard deviation, the greater the differences between the individual determinations and the less the precision of the method.

## 4-6. PERCENT COEFFICIENT OF VARIATION

a. When comparing the results of determinations at different levels of concentration, the standard deviation may be expressed as a percentage of the mean value. This has been known as the **percent coefficient of variation** (%CV), but the preferred term is **relative standard deviation** (RSD). The percent coefficient of variation is employed to give the relative variability of a test procedure.

b. More simply, the lower the coefficient of variation, the less the dispersion of the results around the mean and the more precise the test.

c. Often, it is necessary to compare the relative variation in various types of test results. Since the means and standard deviations of different groups of data may involve numbers of different magnitudes (large values versus small values) or of different units of measure, absolute variation cannot be compared.

d. For example, it may be desirable to compare the precision of a test for the same constituent using a completely different procedure performed in a completely different laboratory or the variation in weights of males and females, the variation of weight with a variation in height, or the salaries of clinical technicians in the United States and some foreign country.

e. The measure of relative variation is expressed as a fraction of the mean, usually as a percentage and is called the coefficient of variation. It is defined mathematically as:

$$\%CV = \frac{SD}{\bar{X}} \times 100$$

where %CV = percent coefficient of variation
SD = standard deviation
$\bar{X}$ = mean value

NOTE: The percent coefficient of variation is always rounded to the accuracy of one decimal place.

## 4-7. ESTABLISHING A QUALITY CONTROL PROGRAM

a. One major criterion of a good clinical laboratory is that the day to day determinations of a given constituent have a satisfactory degree of precision.

b. This can be best checked by analyzing one or more samples of known concentrations each day and comparing the results. These samples should be similar in composition to the regular laboratory specimens and should have a definite, unvarying concentration. This criterion is best met by using a number of commercially available lyophilized control sera.

c. In setting up a quality control program, the control sera are analyzed each day along with the regular specimens. The control sera should have no preferred position in the order of analysis and must be treated exactly like the regular serum in manual methods (presumably this will automatically be done in automated methods).

d. Records are kept of the daily results and values may be plotted on a control graph such as the one in figure 4-2.

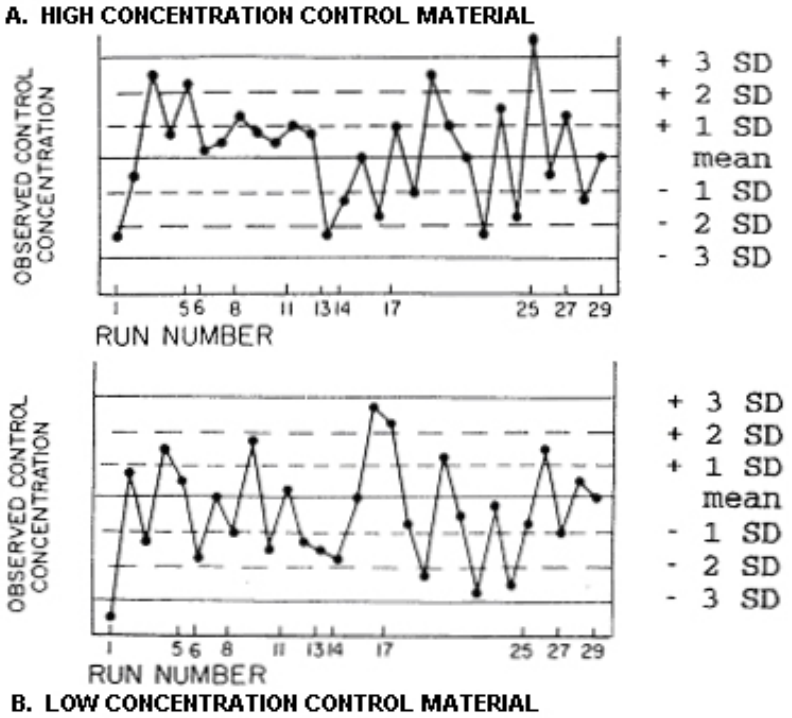



Figure 4-2. Daily records control graph.

NOTE: Gaussian distribution in this case: Arranging the entire month's control values in increasing magnitude along the vertical axis demonstrates that the values are distributed evenly on either side of the mean. So that 68.27% of these values would fall within ±1 standard deviation from the mean, 95.45% of the values fall within ±2 SD, and 99.73% are within ±3 SD of the mean.

## 4-8. CONSTRUCTING AND INTERPRETING A QUALITY CONTROL CHART (LEVEY-JENNINGS)

a. In any quality control program, the technician measures the magnitude of experimental errors and determines acceptable limits.

b. The plotting of daily values on a quality control chart is one of the best ways to graphically follow the accuracy of a test. The mean ($\overline{X}$) control chart for the clinical chemistry laboratory was described in 1950 by Levey and Jennings.

c. The control chart is a plot of the mean value ($\overline{X}$) $\pm$ 2 standard deviations (an extension of normal Gaussian distribution) versus days of the month (thirty days or thirty consecutive observations). It is generally concluded that ideally, for quality control, 30 values must be obtained before attempting to calculate mean and standard deviation for any given constituent.

d. Because of the number of workdays in a month, 20 samples are used to establish the mean in some cases. When control limits for various chemistry procedures have been established, prepare quality control charts reflecting the $\pm$ 2 standard deviation range for each test control values in mg/dl, as shown in the following steps (see figure 4-3).

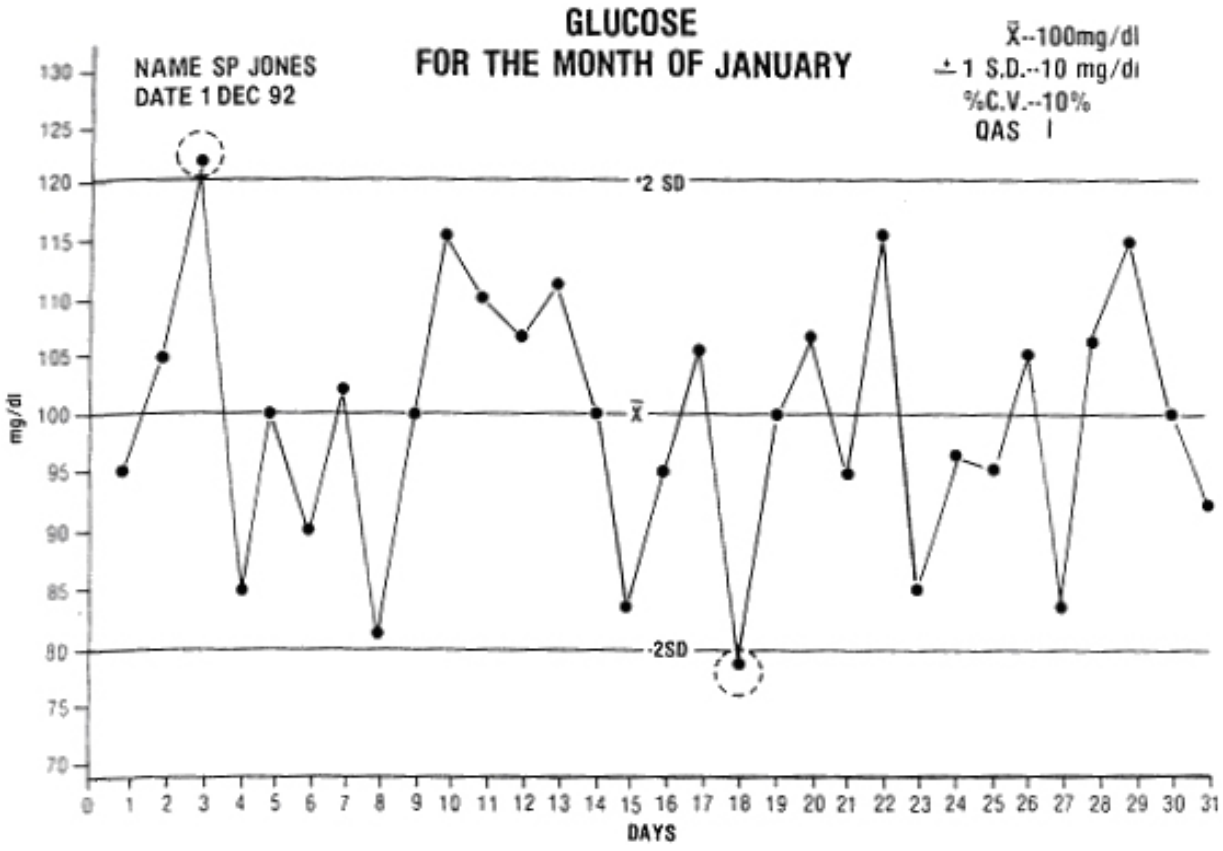



Figure 4-3. Example of a Levey-Jennings plot.

(1) Guide to labeling of the vertical (y) axis.

(a) On the "y" axis, plot control values that represent the mean ± 3 SD.

(b) The mean line is in the center of the chart and labeled as such.

(c) The units are evenly spaced and clearly labeled.

(d) The units of concentration should appear on the y axis.

(e) The graph is broken between zero and the first point.

(2) Guide for labeling of the horizontal (x) axis.

(a) The x axis is divided into 31 even divisions.

(b) The divisions are labeled with the days of the month, from one (1) to thirty-one (31).

(c) The units of this axis are labeled as DAYS.

(3) Guide for plotting of data.

(a) The mean line is in the center of the chart and clearly labeled.

(b) The plus two (2) standard deviation (+ 2 SD) is clearly labeled.

(c) The minus two (2) standard deviation (- 2 SD) line is clearly labeled.

(d) Daily data is plotted by the use of a "dot" and the dots are connected from day to day.

(e) All data (dots) that are out of control (outside ± 2 SD) are circled. Data that falls out of control should be repeated and new data plotted on the chart for that day because a potential problem exists and you must try to determine its cause.

(f) All shifts and trends should be indicated by circling each dot that is involved and labeling it appropriately on the chart (shift or trend).

(4) Guide for title and data blocks.

(a) The chart has the name of the individual that prepared the chart in the upper left corner.

(b) The date of preparation is indicated on the chart below the preparer's name.

(c) The name of the test is indicated at the top center of the chart and directly underneath the month for which the chart is being used.

(d) The mean value $(\bar{X})$ is indicated at the top right of the chart.

(e) The value for $\pm$ 1 SD is indicated at the top right of the chart directly under the mean value.

(f) The percent coefficient of variation (%CV) is the third item in the upper right portion of the chart.

(g) The mean, standard deviation, and percent coefficient of variation must have the correct units of report.

e. The $\pm$ 2 SD represents the allowable **confidence** limits for the control data. This is generally interpreted as the area under a Gaussian curve where 95 percent of the daily control values will fall in a purely random distribution. Approximately one out of twenty test values will fall outside these limits.

(1) Interpreting values that fall outside these limits, as being "out of control," must be exercised with caution. The occasional value that falls outside two standard deviations may or may not be significant.

(2) An initial check of a potential problem would be to repeat the control along with two or three random samples to determine whether the control sample reverts to a value between the limits before reporting any patient results.

(3) If the value returns to normal limits, the chances are that a random error occurred. If more than one value in 20 consecutive values or if two consecutive values occur outside the "confidence limits," a biased condition may exist and the source of a potential problem must be determined.

f. If six consecutive values fall below or above the mean or if six consecutive values fall on the mean, the test system is said to be biased or out of control.

(1) This warrants an examination of the test system to identify and correct any problems. The selection of six values is also arbitrary but is generally accepted as a practical approach in most clinical laboratories.

(2) When conditions other than those measured as experimental errors are observed, such as deterioration of standard and/or reagent, the error usually becomes apparent as a trend or shift in values.

(3) If values continue to increase or decrease for six consecutive days, these represent a "trend."

(a) Upward trends may be caused by a number of different errors such as deteriorated reagent and/or standard or incomplete extraction of samples.

(b) Downward trends are usually caused by the opposite condition.

(4) A "shift" is formed by values of six consecutive values that fall either above or below the mean, but do not touch or cross the mean. If these six consecutive values distribute themselves on one side or the other of the mean but maintain a constant level, (do not continue to rise or fall crossing the mean), the chart is said to have taken a shift.

(a) An upward shift might indicate that a new standard at a higher concentration (improper reconstitution) has been prepared.

(b) Generally, downward shifts are caused by conditions opposite of those causing an upward shift.

## 4-9. WESTGARD MULTI-RULE CHART

Most quality control decisions are made on a daily basis. For some procedures, the controls are tested first. Therefore, there is an immediate identification of problems or systems out of control. For other procedures that are run in a batch process, as with automated techniques, the controls and unknowns are only available at the end of the analytical run. Daily bench level quality control testing can only be used to detect systematic errors and decreases in precision. It cannot detect random errors, which occur unpredictably. Random errors are detected when significantly abnormal results are repeated.

a. A "multi-rule" procedure developed by Westgard et al utilizes a series of control rules for interpreting control data. Error detection is improved by selecting those rules that are particularly sensitive to random or systematic error. The procedures require a chart that can be adapted from an existing Levey-Jennings chart by adding one or two sets of control limits. The procedure requires a chart having lines for control limits drawn at the mean, mean + 1 SD, mean + 2 SD, and mean + 3 SD (see figure 4-4). This is how the control rules are used.

| Run Number | Decision to Run | | Control Rule Violated | | | | |
|---|---|---|---|---|---|---|---|
| | Accept | Reject | $1_{3s}$ | $2_{2s}$ | $R_{4s}$ | $4_{1s}$ | $10_{\bar{x}}$ |
| 1 | | X | X | X | | | |
| 3 | X | | | | | | |
| 5 | X | | | | | | |
| 2 | | X | | | | | X |
| 3 | X | | | | | | |
| 16 | | X | X | | | | |
| 17 | | X | X | X | | | |
| 18 | | X | X | X | X | | |
| 22 | | X | | X | | | |
| 24 | X | | | | | | |
| 25 | | X | X | X | | | |

Figure 4-4. Westgard multi-rule control chart and interpretation.

(1) $1_{2s}$ -- one control observation exceeding $\pm$ 2 SD used as a "warning" rule.

(2) $1_{3s}$ -- one control observation exceeding $\pm$ 3 SD is a "rejection rule" that is primarily sensitive to "random error."

(3) $2_{2s}$ -- two consecutive control observations exceeding the $\pm$ 2 SD limit is a "rejection rule" that is sensitive to "systematic error."

(4) $R_{4s}$ -- one observation exceeding plus 2 SD, and another exceeding the minus 2 SD, is a "rejection rule" that is sensitive to "random error."

(5) $4_{1s}$ -- four consecutive observations exceeding $\pm$ 1 SD is a "rejection rule" that is sensitive to "systematic error."

(6) $10_{\bar{x}}$ ten consecutive observations falling on one side of the mean (above or below) is a "rejection rule" that is sensitive to "systematic error."

b. Using the multi-rule procedure is similar to using a Levey-Jennings chart, but data interpretation is more structured.

(1) Control material should be analyzed for at least 20 different days.

(2) A control chart should be constructed to include data for $\pm$ 4 SDs. There should be three distinct separations from the mean, mean $\pm$ 1 SD, mean $\pm$ 2 SD, and mean $\pm$ 3 SD.

(3) At least two control specimens should be introduced into the analytical run. Typically this involves a normal concentration and an abnormally high concentration for the test methodology. Record the control results and plot on its respective control chart.

(4) Inspect the control data using Westgard control rules:

(a) When all of the rules indicate the run is in control, accept the analytical run and report results.

(b) When both control observations fall within the 2 SD limit, the run is accepted and the results are reported.

(c) When one of the control observations exceeds the 2 SD limits, hold the results.

(d) When any one of the rules indicates the run is out of control, reject the analytical run and do not report the results.

(5) When an analytical run is out of control, a determination of the type of error occurring based on the control rules should be made. Look for the sources of the errors. Correct the problem and if possible, rerun the entire test--both patient and control samples.

## 4-10. QUALITY CONTROL REVIEW

a. In summary, each laboratory is responsible for defining its own system of quality control decision. Quality control includes: comparing the probability for error detection between Westgard multi-rule and Levey-Jennings charts, having 3 SD limits, showing improved error detection in the multi-rule procedure.

b. In conclusion, an experienced employee well informed on quality control principles and procedures should be assigned to direct and monitor the quality control program. An effective quality control program for clinical chemistry requires attention, interest, and consistency of application on the part of all assigned personnel. Quality control, then, benefits the laboratory in many important ways. It serves as an objective guide upon which we can judge the precision and accuracy of laboratory results; it functions as a warning system by letting us know when a particular test procedure is becoming less accurate; and it establishes confidence limits useful in diagnosis.

**Continue with Exercises**

## EXERCISES, LESSON 4

**INSTRUCTIONS:** Answer the following exercises by marking the lettered response that best answers the question by completing the incomplete statement, or by writing the answer in the space provided at the end of the question.

After you have completed all the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Which statement below correctly describes quality control?

   a. The standard used is an unknown value or composition and based upon an established procedure.

   b. The control is a known substance.

   c. Control specimens may vary in composition and are used as standards.

   d. The standard is a known composition, the value of which is established by an analytical procedure different from the one used in the clinical laboratory.

2. Select the definition that best defines quality control.

   a. A system by which you can determine the reproducibility of a laboratory procedure.

   b. A procedure by which you can guarantee 100% on all laboratory tests.

   c. A system used to ensure that only the tests needed by a patient are performed.

   d. A set of procedures which are used to reduce the costs of laboratory tests.

3. Select the definition of the term "precision."

    a. Precision refers to the closeness of a test result to each other.

    b. Precision refers to how close the value of a determination is to the actual valve of a specimen.

    c. Precision is a quality control term associated with the mean of a set of data.

    d. Precision refers to the length of time data has been collected.

4. "Accuracy" is defined as:

    a. The acceptable range for a test procedure.

    b. The closeness of a test result to each other.

    c. The closeness of a test result to the true value.

    d. Freedom of variation.

5. Improper labeling or storing reagents and incorrectly typing the container and storage temperature are examples of:

    a. Errors frequently resulting from failing to observe basic precautions and laboratory rules.

    b. Major applications currently in use or internal and external quality control systems.

    c. Communication errors.

    d. Static control techniques.

6. Internal control systems use what type of samples?

    a. Samples of unknown concentration.

    b. Pooled serum samples.

    c. Manufactured assayed standards.

    d. Samples of known analytic content.

7. What is the major application for an external quality control system?

    a. Provide precision statistics only.

    b. Test the individual laboratories precision.

    c. Provide periodic benchmark accuracy or bias estimates to individual laboratories.

    d. Assuring quality of total laboratory performance.

8. Which one of the common errors needs closer scrutiny because few laboratory workers pay much attention to it?

    a. Basic mathematical skills.

    b. Matrix.

    c. Transcription.

    d. Contamination of specimens.

9. To help reduce the common errors noted in exercise 8, what method(s) should be used to detect this problem?

    a. Train technicians to do it right the first time.

    b. Develop/post a list of frequently used drugs and laboratory procedures that might interfere with obtaining desired results. Have everyone read it.

    c. Use the Levey-Jennings chart.

    d. Use larger random variations in measurement.

10. A quality control objective is to eliminate common errors. Which answer will accomplish the most to reduce these errors?

    a. Properly identify patient and/or specimen.

    b. Properly label or store reagents.

    c. Transcribe correct reporting results.

    d. All of the above.

11. Even with routine quality assurance efforts, errors in analysis may still occur with the use of:

    a. Standards.

    b. Control specimens.

    c. Proficiency testing.

    d. Other aspects of process control.

    e. a and c.

    f. All of the above.

12. Even with routine quality assurance efforts, errors may occur in the specimen matrix factor or interpretation because of an unexpected:

    a. Physiologic or genetic factor.

    b. Drug.

    c. Dietary component.

    d. a, b, and c.

    e. Environmental factor.

    f. All of the above.

13. Which of the following statements is correct concerning tabular records quality control in clinical chemistry?

    a. Tabular records with appropriate calculations can be used to implement the techniques.

    b. Tabular records are easier to interpret than graphical displays.

    c. Tabular data readily reveals subtle changes that may be occurring with an analytical method.

    d. All of the above.

14. Which chart is the most widely used technique in clinical chemistry?

    a. Westgard multi-rule chart.

    b. Levey-Jennings chart.

    c. a and b.

    d. None of the above.

15. Select the correct formula used to determine the mean or arithmetic average?

    a. $\overline{X}\,(\text{mean}) = \dfrac{X_1 - X_2 + X_3 + X_n}{n}$

    b. $\overline{X}\,(\text{mean}) = \dfrac{X_1 + X_2 - X_3 + X_n}{n}$

    c. $\overline{X}\,(\text{mean}) = \dfrac{X_1 + X_2 + X_3 - X_n}{n}$

    d. $\overline{X}\,(\text{mean}) = \dfrac{X_1 + X_2 + X_3 + X_n}{n}$

16. What number of observations must be obtained if the mean, and deviations to be measured from it, is to have any significance?

    a. 5 or more.

    b. 10 or more.

    c. 15 or more.

    d. 20 or more.

    e. Less than 5.

17. If fewer than 20 observations are collected to obtain the mean, will our results be correct? If not, why not?

    a. The results will be correct.

    b. We have no way of knowing if our results are misleading, due to unusually large random variations in measurement.

    c. We will know the results are misleading because of the constant large random variations in measurement.

    d. None of the above.

18. Using this set of numbers 41.0, 37.01, 42.35, and 41.65 calculate the mean.

    a. 38.

    b. 40.5.

    c. 41.66.

    d. 42.72

19. Using this set of numbers 23.2, 34.01, 22.25, and 24.55 calculate the mean.

    a. 21.74.

    b. 22.33.

    c. 23.33.

    d. 26.00.

20. Select the response below that is the mean of the following numbers: 24, 36, 40, 32, 28, 74, and 26.

    a. 26.54.

    b. 40.5.

    c. 32.45.

    d. 37.14.

21. Select the response below that is the mean of the following numbers: 9, 10, 12, 13, 15, 11, and 14.

    a. 9.

    b. 10.

    c. 11.

    d. 12.

22. The mathematical formula for standard deviation (SD) of a number of values is:

a. $$SD = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 1}}$$

b. $$SD = \sqrt{\frac{\text{Sum of mean differences}}{\text{Number of values} - 1}}$$

c. $$SD = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 2}}$$

d. $$CV = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 3}}$$

e. $$CV = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} \pm 1}}$$

23. Which statement(s) best describe(s) the mathematical computation to determine the standard deviation?

a. Calculate the average value of the determinations $(\overline{X})$, finds the difference between the separate values of ($X_i$), squares these differences $(\overline{X} - X_i)^2$, and finds the sum of these squares (Σ indicates the summation).

b. This sum is then divided by one less than the number of values (N - 1) and the square root of the quotient is extracted.

c. Round off one standard deviation to one more decimal place than the data set and two and three standard deviation ranges to the same accuracy as the data set.

d. All of the above.

24. Select the response below that is one standard deviation of the following numbers: 4, 5, 6, 7, and 8.

    a. 10.

    b. 6.

    c. 2.5.

    d. 1.6

25. The range of dispersion or distribution of values about their mean is called:

    a. Variation.

    b. Standard deviation.

    c. Coefficient of variation.

    d. Quality control criterion.

26. Which statement is correct concerning standard deviation?

    a. The greater the standard deviations, the greater the differences between the individual determinations and the less the precision of the method.

    b. The lesser the standard deviations, the greater the differences between the individual determinations and the less the precision of the method.

    c. The greater the standard deviations, the greater the differences between the individual determinations.

    d. When determinations number 20 or more and are calculated, approximately 68 percent of all values will fall within $\pm$ 2 SD from the mean.

27. If the standard deviation (SD) is 0.2 and the mean is 1.4, what is the percent coefficient of variation?

    a. 0.1429

    b. 1.4

    c. 14%

    d. 14.3%

28. Which statement is correct concerning the percent coefficient of variation?

    a. When comparing the results of determinations at different levels of concentration, the standard deviation may be expressed as a percentage of the mean value.

    b. Although known as the percent coefficient of variation (%CV), the preferred term is relative standard deviation (RSD).

    c. The higher the coefficient of variation, the less the dispersion of the results around the mean and the more precise the test.

    d. a and b.

    e. a and c.

29. When setting up a quality control program, how must control sera be treated?

    a. Better than the regular serum.

    b. Not as carefully as the regular serum.

    c. Exactly like the regular serum in manual methods.

    d. Exactly like the regular serum using automation methods.

30. It is generally concluded that ideally _____values must be obtained prior to attempting to calculate mean and standard deviations used to construct a Levey-Jennings chart.

    a. 10.

    b. 20.

    c. 30.

    d. 37.

31. In figure 4-3, on which day does the glucose level fall below 80 mg/dl on the Levey-Jennings plot chart?

    a. 4 Dec.

    b. 8 Dec.

    c. 18 Dec.

    d. 27 Dec.

32. What does the vertical (y) axis represent on the Levey-Jennings plot chart?

    a. Days of the month, from one to thirty-one.

    b. 31 even divisions.

    c. Guide for plotting of data.

    d. Control concentration values plotted over $\pm$ 3 SD about the mean.

33. Which statement is correct concerning guides for plotting the Levey-Jennings quality control chart?

    a. The mean line is in the center of the chart and clearly labeled.

    b. The plus three (3) standard deviation (+ 3 SD) is clearly labeled.

    c. The minus four (4) standard deviation (- 4 SD) line is clearly labeled.

    d. Daily data is plotted by the use of a "square" and the squares are connected from day to day.

34. When plotting quality control data on the Levey-Jennings chart, six consecutive days fall above the established mean but do not touch or cross the mean, this is termed a:

    a. Shift.

    b. Trend.

    c. Shift and control.

    d. Mistake and the mean must be recalculated.

35. When labeling a Levey-Jennings quality control chart, the "x" axis should be labeled:

    a. Concentration.

    b. Month.

    c. Days.

    d. Test Procedure.

36. The name of the preparer should be placed where on a Levey-Jennings quality control chart?

    a. Centered under the Title.

    b. Preparer's name is not required.

    c. Upper left corner.

    d. Upper right corner.

37. The Westgard multi-rule chart differs from the Levey-Jennings chart in that it:

    a. Uses a series of control rules for interpreting control data but the interpretation is more structured.

    b. Immediately identifies problems or systems out of control.

    c. a and b only.

    d. Is an improved system to detect errors but only if they are systematic errors.

    e. Needs 1 or 2 lines or set of control limits to be added at the mean, mean $\pm$ 1 SD, mean $\pm$ 2 SD, and mean $\pm$ 3 SD.

    f. a, b, d, and e.

38. When using a Westgard multi-rule chart, you observe that one control result fell outside 2 SD limits. What should be done?

    a. Run is accepted and results reported.

    b. Reject the entire run and do not report any of the results.

    c. Report all results and repeat only the control that is out of 2 SD limits.

    d. Hold the results until the error is determined.

39. What is the rejection rule when four consecutive observations exceed $\pm$ 1 SD using a Westgard multi-rule chart?

    a. $4_{1s}$ -- measures "systematic error."

    b. $R_{4s}$ -- measures "random error."

    b. $1_{3s}$ -- measures "random error."

    c. $1_{2s}$ -- measures "warning" rule.

    d. $10_{\bar{x}}$ -- measures "systematic error."

40. What is the significance of an "X" being shown under the $10_{\bar{x}}$ in the Westgard multi-rule chart at figure 4-4?

    a. One control observation exceeding $\pm$ 3 SD is a rejection rule that is primarily sensitive to random error.

    b. Two consecutive control observations exceeding the $\pm$ 2 SD limit is a rejection rule that is sensitive to systematic error.

    c. Ten consecutive observations falling on one side of the mean (above or below) is a rejection rule that is sensitive to systematic error.

    d. The interpretation is incorrect.

41. The multi-rule procedure is considered to be similar to the Levey-Jennings chart except that the interpretation is:

    a. Easy.

    b. Structured.

    c. Systematical.

    d. Accurate.

42. How many distinct separations from the mean, mean ± 1 SD, mean ± 2 SD, etc., is the multi-rule chart to have?

    a. 1.

    b. 2.

    c. 3.

    d. 4.

43. When an analytical run is out of control and the type of error determined, what are the next procedures in sequence?

    a. Look for the source of the error, correct the problem, if possible, and rerun the entire test--both patients and controls.

    b. Look for more error sources and rerun the entire test--both patients and controls.

    c. Correct the problem, if possible, and rerun the patient tests.

    d. a and b.

44. When inspecting the control data using Westgard control rules, what happens when both control observations fall within the 2 SD limit?

    a. The run is not accepted but the results are reported.

    b. The run is accepted and the results are not reported.

    c. The run is accepted and the results are reported.

    d. Neither the run is accepted nor the results reported.

45. What do you do when any one of the Westgard control rules indicate the run is out of control?

    a. Make no report of the results but accept the analytical run.

    b. Do not accept the analytical run but do report the results.

    c. Accept the analytical run and report the results.

    d. Reject the analytical run and do not report the results.

46. Each laboratory quality control decision should include:

    a. Comparing the probability for error detection between Westgard multi-rule and Levey-Jennings charts.

    b. Having 3 SD limits.

    c. a and b.

    d. Showing improved error detection in the multi-rule procedure.

    e. All of the above.

47. What are the makeup and benefits of a good quality control program? It:

    a. Serves as an objective guide upon which we can judge the precision and accuracy of laboratory results.

    b. Functions as a warning system by letting us know when a particular test procedure is becoming less accurate.

    c. Establishes confidence limits useful in diagnosis.

    d. All of the above.

48. Who should be assigned to direct and monitor the quality control program?

    a. A technician.

    b. A programmer.

    c. An experienced employee well informed on quality control matters.

    d. None of the above.

**Check Your Answers on Next Page**

**SOLUTIONS TO EXERCISE, LESSON 4**

1. d (para 4-1b(1))
2. a (para 4-1b)
3. a (para 4-1b(2))
4. c (para 4-1b(1)(2))
5. a (para 4-2a)
6. d (para 4-1c)
7. c (para 4-1c)
8. b (para 4-2c)
9. b (para 4-2d)
10. d (para 4-2a)
11. f (para 4-2b)
12. f (para 4-2b)
13. a (para 4-3)
14. b (para 4-8(b))
15. d (para 4-4a)
16. d (para 4-4b)
17. b (para 4-4b)
18. b (para 4-4a)
19. d (para 4-4a)
20. d (para 4-4a)

Step 1: Find the sum of all the numbers.
Solution: 24 + 36 + 40 + 32 + 28 + 74 + 26 = 260
Step 2: Divide the sum by the number of numbers present. 260/7 = 37.14

21. d (para 4-4a)

22. a (para 4-5b)

23. d (para 4-5b)

24. d (para 4-5b)

Solution - Step 1: Prepare Table.

| Number | Mean | Mean - Number | (Mean Number)$^2$ |
|---|---|---|---|
| 4 | 6 | 2 | 4 |
| 5 | 6 | 1 | 1 |
| 6 | 6 | 0 | 0 |
| 7 | 6 | -1 | 1 |
| 8 | 6 | -2 | $\frac{4}{10}$ = Sum of squared differences from mean |

Step 2: Use the formula.

$$SD = \sqrt{\frac{\text{Sum of squared differences from mean}}{\text{Number of values} - 1}}$$

$$SD = \sqrt{\frac{10}{5-1}} = \sqrt{\frac{10}{4}} = \sqrt{2.5} = 1.58 \text{ or } 1.6$$

Step 3: One standard deviation should be rounded to one more decimal place than the data set.

25. b (para 4-5c)

26. a (para 4-5c)

27. d (para 4-6)

Step 1: $\frac{0.2}{1.4} \text{ X } 100 = 14.2857$ or 14.3%

Step 2: Percent coefficient of variation is always rounded to the accuracy of one decimal place.

28. d (para 4-6a)

29. c (para 4-7c)

30. c (para 4-8c)

31. c (Figure 4-2)

32. d (para 4-8d(1))

33. a (para 4-8d(3))

34. a (para 4-8f(4))

35. c (para 4-8d(2))

36. c (para 4-8d(4))

37. f (para 4-9, 4-9a)

38. d (para 4-9b(4))

39. a (para 4-9a(5))

40. c (para 4-9a(6), Figure 4-4)

41. b (para 4-9b)

42. c (para 4-9b(2))

43. a (para 4-9b(5))

44. c (para 4-9b(4)(b))

45. d (para 4-9b(4)(d))

46. e (para 4-10a)

47. d (para 4-10b)

48. c (para 4-10b)

**End of Lesson 4**

## LESSON ASSIGNMENT

**LESSON 5** Introduction to Organic Chemistry.

**TEXT ASSIGNMENT** Paragraphs 5-1 through 5-12.

**LESSON OBJECTIVES** After completing this lesson, you should be able to:

5-1. Select the statement that best defines the following terms: aliphatic, aromatic, saturated, unsaturated, isomer, and covalent bond.

5-2. Select the correct class of organic compounds to which it belongs from the structural formula.

5-3. Select the appropriate name of an organic compound using IUPAC or common nomenclature from the structural formula.

5-4. Select the correct structural formula from the IUPAC or common name of an organic compound.

5-5. From a class of organic compounds, select the correct chemical reaction(s) the group will undergo and the product(s) that will be formed.

**SUGGESTION** After studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

# LESSON 5

# INTRODUCTION TO ORGANIC CHEMISTRY

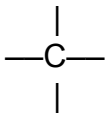
## Section I. INTRODUCTION TO BASIC CONCEPTS

### 5-1. SIGNIFICANCE OF ORGANIC CHEMISTRY

Organic chemistry was first used as a term to designate those chemical compounds produced by living cells. Most compounds produced by cells contain the element carbon. This caused organic chemistry to be redefined as the branch of chemistry, which deals with carbon compounds. Even those compounds with no relationship to life are placed in this branch. Inorganic chemistry includes all other substances that do not contain carbon. It may seem strange that in this division, organic chemistry deals with one element, carbon, while the other division contains the rest of the elements. The reason for this is that organic compounds far outnumber inorganic compounds. In fact, out of the millions of known compounds, only about 500,000 are inorganic.

### 5-2. CARBON AND BASIC ORGANIC STRUCTURES

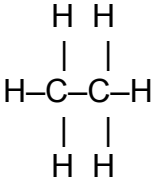
The carbon atom has four electrons in its outermost shell, all of which are available for the formation of chemical bonds with other elements or additional carbon atoms. Carbon is an element that does not ionize; gain or lose electrons through the transfer of electrons from one atom to another. When carbon forms bonds, it shares its electrons with other atoms. This type of bond, in which electrons are shared, is called a covalent bond. In most organic compounds, each carbon forms four covalent bonds. Carbon also is unique in that it is the only element that can form bonds between itself in long chains, branched chains, or in a cyclic (ring) structure.



a. **Structural Formulas.**

(1) Organic compounds can be represented or written with the use of structural, condensed structural, or molecular formulas. Structural formulas represent the atoms of elements in a compound and the bonds that hold them together. The covalent bonds are represented by the dashes between atoms.

Ethane ($CH_3CH_3$)



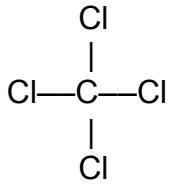
(2) The condensed structural formula is an abbreviated form in which some of the bonds are not represented. The condensed structural formula for ethane is $CH_3CH_3$. A molecular formula represents the number and type of atoms in the compound, but not the arrangement or relationship of the atoms to each other. The molecular formula for ethane is $C_2H_6$. Many organic compounds will have different structural (regular or condensed) formulas but have the same molecular formula. These compounds are called isomers. For example, ethanol is an isomer of dimethyl ether. They both have the molecular formula $C_2H_6O$, but their structural formulas differ.

| | |
|---|---|
| $CH_3CH_2–OH$ | $CH_3–O–CH_3$ |
| Ethanol | Dimethyl ether |

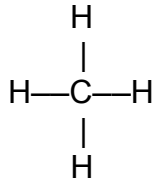
b. **Carbon Bonding**.

(1) It has been stated that carbon will form four covalent bonds. For example, in the structural formula ethane, note that each carbon is surrounded by four bonds (dashes). These are called **single bonds** and represent the sharing of one pair of electrons between the carbon atoms and carbon to hydrogen atoms. Group VIIA elements (halogens), such as chlorine (Cl) and fluorine (F), will also form single bonds with carbon. The following are some examples of simple structural formulas where a single unit of carbon (C) unites with an element that is **univalent** to form only single bonds.

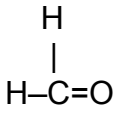
Carbon tetrachloride ($CCl_4$)



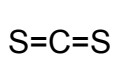
Methane ($CH_4$)



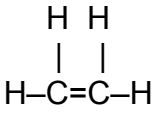
(2) Carbon also combines with **divalent** and **trivalent** elements. These elements will form double and triple bonds respectively. Oxygen and sulfur are elements that are capable of forming bonds where two pairs of electrons are shared with carbon. This type of bond is called a **double bond** and is represented by two dashes. Carbon also can form double bonds with other carbons.



Methanal ($CH_2O$)

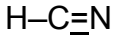


Carbon disulfide ($CS_2$)

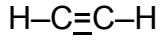


Ethene ($C_2H_4$)

(3) Nitrogen and other carbons can form bonds in which three pairs of electrons are shared and they are called **triple bonds**.



Hydrogen cyanide

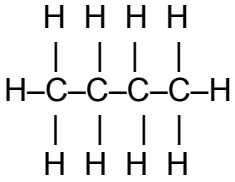


Ethyne

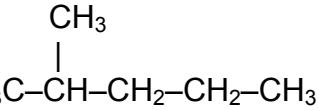
NOTE: In the examples of **double** and **triple bonds**, each carbon still forms a total of four bonds.

c. **Chains of Carbon Atoms**. Since carbon atoms can unite with each other, combinations of these atoms, within each other, can result in chains of atoms of widely varying lengths. Branched chains are also common.

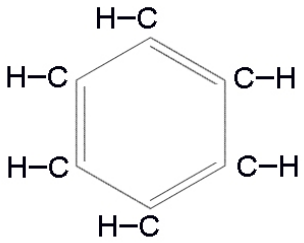
Butane ($C_4H_{10}$) (open chain)



2–methyl pentane



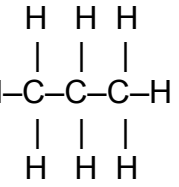
d. **Rings of Carbon Atoms**. Rings also can result because carbon atoms bond with each other; the difference between rings and chains is that the C atoms in a ring share electrons with each other in a closed-circuit arrangement.



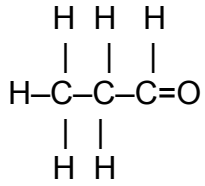
Benzene ($C_6H_6$)

e. **Saturated Compounds.** A saturated organic compound is one in which the combining capacities of all the carbons are satisfied. Bonds that connect carbon atoms to other carbon atoms in saturated compounds (e.g., ethane) will always be single bonds. This is because the carbon atoms are sharing the minimum number of electrons to bind to each other and allowing for three additional bonds to be formed with other carbons or non-carbon atoms. The remaining bonds to non-carbon atoms may be single, double, or triple bonds. Propanal is a saturated organic compound because all the carbon to carbon bonds are single bonds.

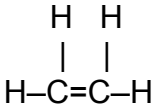
Propane ($C_3H_8$)



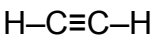
Propanal ($C_3H_6O$)



f. **Unsaturated Compounds**. In an unsaturated organic compound, at least two carbon atoms are joined by a double or triple bond (refer to examples ethene and ethyne). The term, unsaturated, implies that other atoms could be bound with these carbon atoms, thereby making new compounds. Unsaturated compounds are more chemically active than saturated compounds because double and triple bonds are less stable than single bonds.

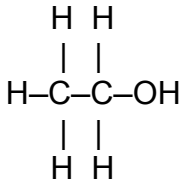


Ethene [Ethylene ($C_2H_4$)]

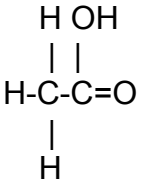


Ethyne [Acetylene ($C_2H_2$)]

g. **Functional Groups**. Organic chemistry is made simpler in that reactions involving organic compounds seldom involve the whole molecule. Only one small portion of a molecule is usually involved, which is called the functional group. This group may be a specific type of bond, an atom that has replaced hydrogen, or a radical (groups of atoms that act as a single atom).



Ethanol ($C_2H_5OH$)

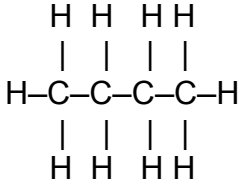


Ethanoic acid ($CH_3COOH$)

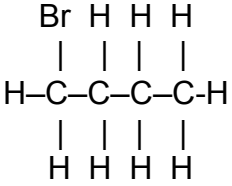
NOTE: Notice that these structural formulas have been written to indicate the functional group (–OH and –COOH). Ethanol can be written two ways, either as $C_2H_5OH$ or $CH_3CH_2OH$. The latter indicates that ethanol is a derivative of ethane ($CH_3CH_3$), in which one of the hydrogen atoms has been replaced by the radical –OH. By substituting –COOH in place of hydrogen in methane ($CH_4$), ethanoic acid is formed.

h. **Divisions of Organic Compounds**.

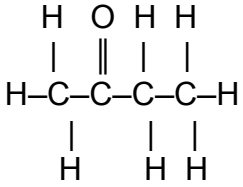
(1) Aliphatic compounds. These compounds are organic compounds in which the molecules are composed of open or branched chains of carbon atoms (saturated or unsaturated) to which atoms or radicals are attached.



Butane
($CH_3(CH_2)_2CH_3$)



1–Bromobutane
($CH_2Br(CH_2)_2CH_3$)



2–Butanone
$CH_3–C–CH_2CH_3$)

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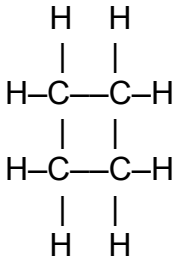
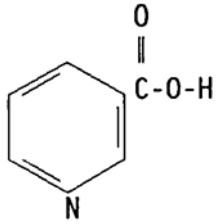
(2) Carbocyclic compounds. These are organic compounds which contain rings of carbon atoms.

Cyclobutane
($CH_2CH_2CH_2CH_2$)

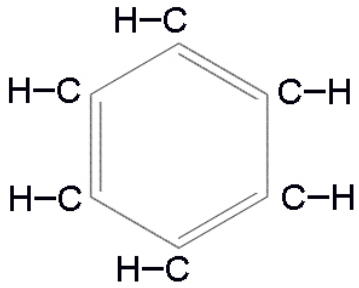


(3) Heterocyclic compounds. These carbocyclic compounds have some other element in addition to carbon in the ring.

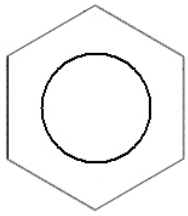



Nicotinic acid ($C_5H_4NCOOH$)

(4) Aromatic compounds. Benzene, a carbocyclic compound, and many of its derivatives have an aromatic odor, which causes members of this series to be often called the aromatic compounds.




Benzene ($C_6H_6$)



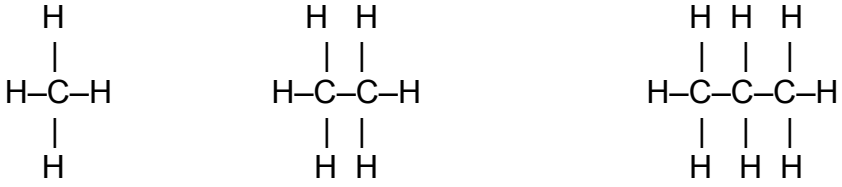
Shorthand structure of benzene.

## Section II. CLASSES OF ORGANIC COMPOUNDS

### 5-3. HYDROCARBONS

Hydrocarbons are compounds containing only carbon and hydrogen.

a. **Alkanes**. Alkanes are saturated aliphatic compounds which may be considered to be derivatives of methane, the simplest member of the group. When reviewing the structural formulas below, note that each compound will differ from the one preceding it by only one carbon and two hydrogens ($CH_2$). Longer or highly branched alkanes can be formed by the addition of more $CH_2$ units.

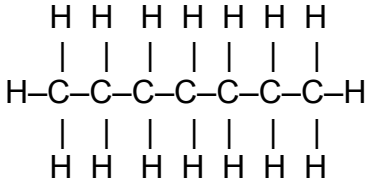


| Methane | Ethane | Propane |
|---|---|---|
| ($CH_4$) | ($CH_3CH_3$) | ($CH_3CH_2CH_3$) |

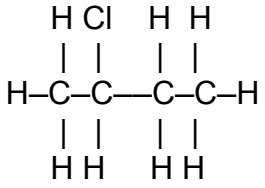
(1) IUPAC nomenclature. The International Union of Pure and Applied Chemistry (IUPAC) has devised a standardized method for naming the unlimited number of structurally and chemically different organic compounds.

(a) The first step in naming an alkane is to identify the longest unbroken chain of carbon atoms. The number of carbons in the chain will be denoted in the name of the compound by the use of prefixes: 1 carbon is meth-, 2 carbons is eth-, 3 is prop-, 4 is but-, 5 is pent-, 6 is hex-, 7 is hept-, 8 is oct-, 9 is non-, and 10 carbons is dec-.

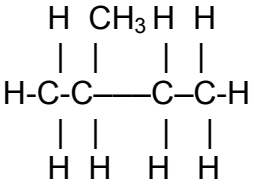
(b) To indicate that the compound is an alkane, the suffix-ane is added to the prefix. For example, a seven carbon long straight chain compound that has only carbon to carbon single bonds is called **heptane**.



(c) The positions of alkyl groups (alkane derivatives) or halogens which have replaced one or more hydrogens in an alkane are identified by using a numbering system.



2–chlorobutane

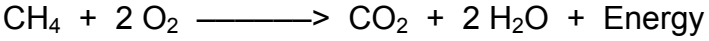


2–methyl butane

NOTE: Notice that the chlorine atom and the methyl group ($CH_3$) are attached to the second carbon of the alkane chain when reading left to right.

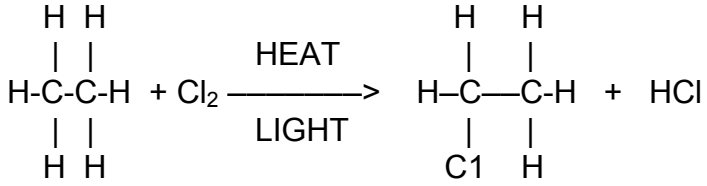
(2) Reactions.

(a) Alkanes have limited reactivity primarily due to the stability of the saturated carbon to carbon bonds. Many of the commonly known alkanes are popular combustible fuels and readily react with oxygen to form carbon dioxide, water, and energy. This type of reaction is known as **combustion**.



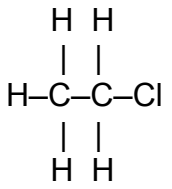
$$CH_4 + 2\,O_2 \longrightarrow CO_2 + 2\,H_2O + \text{Energy}$$

(b) The halogens, fluorine, chlorine, iodine, and bromine will react under vigorous conditions with hydrogen to form halogenated alkanes and acid. This type of reaction in which hydrogen is replaced by a halogen is called a **substitution** reaction.



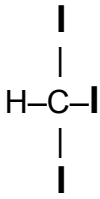
Ethane + Chlorine → Chloroethane Hydrochloric acid

(c) Chloroethane (Ethyl chloride). At room temperature, ethyl chloride is a gas. When it is placed in a container under pressure, it liquefies. By releasing the pressure, a spray of ethyl chloride can be directed against the skin. This spray evaporates rapidly and freezes the area of skin, producing local anesthesia, and has been used for minor surgical procedures such as the lancing of boils.



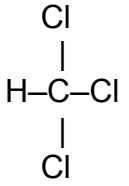
Ethyl chloride ($CH_3CH_2Cl$)

(d) Iodoform. Iodoform is a yellow solid having a characteristic odor. When ethyl alcohol is treated with iodine in the presence of an alkali, iodoform is produced. Iodoform indicates the presence of a form of alcohol more complex than plain methanol.



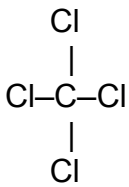
Iodoform ($CHI_3$)

(e) Trichloromethane (Chloroform). Chloroform is an oily liquid. It is colorless and has a characteristic odor. It was once used as an anesthetic, but because it has a toxic effect on the heart and liver, it is no longer used. It has the decided advantage of being non-flammable. It is widely used as a solvent for fats and fat like substances in many chemistry procedures.



Chloroform ($CHCl_3$)

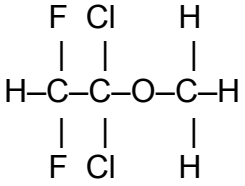
(f) Tetrachloroethane (Carbon tetrachloride). Carbon tetrachloride is a eavy colorless liquid which does not burn. It is an excellent solvent for fats and grease. It has been used extensively in dry cleaning. Because carbon tetrachloride also acts as a liver poison, extreme caution must be used when handling this substance.



Carbon tetrachloride ($CCl_4$)

(g) Penthrane. The most significant property of halogenated hydrocarbons is that as you increase the number of halogens on the compound, the flammability of the compound decreases. This property has been used to produce ethers, which are nonflammable, which may be used as general anesthetics such as:

Methoxyflurane (Penthrane$^{R}$)



b. **Hydrocarbon Radicals**. These may be thought of as hydrocarbons that have lost one or more hydrogen atoms. Because organic radicals have unpaired electrons, they do not exist free but only in chemical union with other radicals or atoms. This fact also applies to hydrocarbon radicals. The methyl radical ($CH_3$) is found in compounds like methyl chloride ($CH_3Cl$) and methyl alcohol ($CH_3OH$). The ethyl radical ($CH_3CH_2$) occurs in compounds such as ethyl bromide ($CH_3CH_2Br$) and ethyl alcohol ($CH_3CH_2OH$).

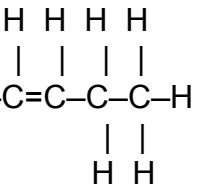
c. **Alkenes**. Alkenes are compounds that contain at least one carbon-to-carbon double bond.

(1) IUPAC nomenclature. The naming of alkenes follows some of the same rules used in naming the alkanes.

(a) Identify the longest carbon chain; which includes the double bond; and write the appropriate prefix. The numbering system is used to identify which carbon (lowest number) is attached to the double bond.

(b) Add the suffix -ene to indicate the compound is an alkene. For example, a five carbon long, straight chain, with a double bond between the second and third carbon is called 2-pentene.

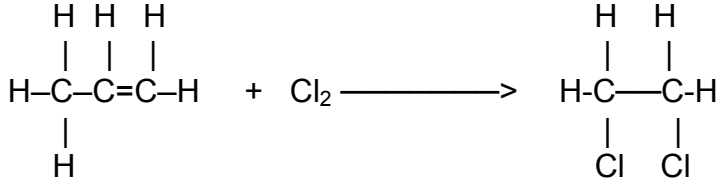
2–Pentene
($CH_3CHCHCH_2CH_3$)



(c) Alkyl groups or halogens attached to alkenes will be identified by the numbering system. Greek prefixes (e.g., di-, tri-) are used to identify multiples of the same alkyl groups or halogens.

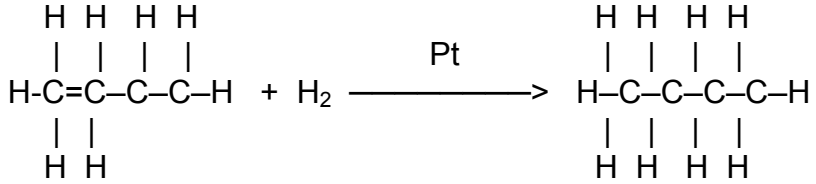
(2) Reactions. Alkenes, like alkanes, will burn in the presence of oxygen (combustion) to yield carbon dioxide, water, and energy. Other reactions involving the alkenes occur at the double bond. The double bond is broken and a pair of electrons becomes available for the formation of bonds with halogens, hydrogen, or hydroxyl group ($OH^-$). These reactions are called **addition reactions**.

(a) Halogenation. In this reaction, halogens are added to an alkene without the formation of an acid. The resulting product is an alkene with a halogen atom attached to each carbon which shared a double bond.



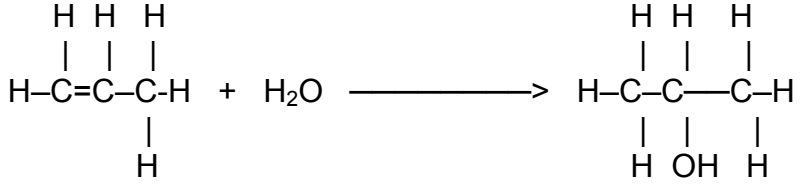
Propene + Chlorine 1,2 Dichloroethane

(b) Hydrogenation. This is similar to halogenation, except hydrogen is the reactant added to the double bond. A catalyst (such as platinum) is required for the resulting product, alkane.



1–Butene + Hydrogen Butane

(c) Hydration. This is an addition reaction in which a hydroxyl group is added to one of the carbons, sharing a double bond. The hydroxyl group is usually added to the carbon, which forms the most bonds with the other carbons, resulting in an **alcohol**.



1–Propene + Water 2–Propanol

NOTE: Notice the hydroxyl group was added to the second carbon, rather than the first, because the second carbon forms two carbon to carbon bonds and the first carbon from only one.

d. **Alkynes**. Alkynes are unsaturated hydrocarbons, which contain at least one carbon to carbon triple bond.

(1) IUPAC nomenclature. The naming of alkynes follows the same rules for naming alkenes. The suffix –yne is added to identify them as alkynes. For example, a two carbon chain with a triple bond between the carbons is called ethyne (acetylene).

Ethyne
(CHCH) H–C≡C–H

(2) Reactions. Alkynes will be involved in the same reactions as the alkenes with comparable formed products.

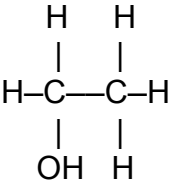
## 5-4. ALCOHOLS

Alcohols are hydrocarbon derivatives in which one or more of the hydrogens in the hydrocarbons are replaced by the hydroxyl ion ($OH^-$ ).

a. **IUPAC Nomenclature.** Alcohols are named utilizing the established rules for naming the alkanes. The suffix –ol is used to indicate that the compound is an alcohol. The longest carbon chain must include the carbon(s) bound to the hydroxyl ion(s). For example, a two carbon compound, with a hydroxyl attached to the first carbon, is called ethanol.

NOTE: The numerical designation 1–ethanol is not necessary, since either carbon can be the number 1 carbon. Counting from left or right is determined by the lowest numerical value, which can be assigned to the hydroxyl ion; the functional group. This applies to all functional groups.

Ethanol
($CH_3CH_2OH$)

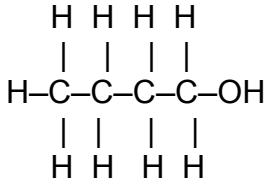


b. **Types of Alcohols**.

(1) Monohydric alcohols. Monohydric alcohols contain only one hydroxyl group per molecule. These are further classified as **primary**, **secondary**, and **tertiary** alcohols.

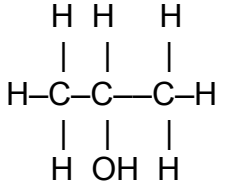
(a) Primary alcohol. If the hydroxyl ion is attached to the terminal carbon, the compound is a primary alcohol. A terminal carbon is a carbon that shares a bond with only one other carbon.

1-Butanol
($CH_3CH_2CH_2CH_2OH$)



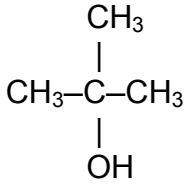
(b) Secondary alcohol. When the carbon atom, to which the hydroxyl group is attached, is bonded to two other carbon atoms, the compound is a secondary alcohol.

2–Propanol
($CH_3CHOHCH_3$)



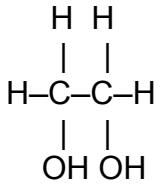
(c) Tertiary alcohol. When the hydroxyl group is attached to a carbon, which also forms bonds with three other carbons, it is a tertiary alcohol.

2–Methyl–2–Propanol



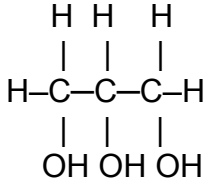
(2) Dihydric alcohols. Dihydric alcohols contain two hydroxyl groups per molecule. The suffix –diol indicates that the compound is an alcohol with two hydroxyl functional groups.

1,2 Ethanediol
($CH_2OHCH_2OH$)



(3) Trihydric alcohol. Trihydric alcohols contain three hydroxyl groups per molecule. The suffix –triol indicates that the compound is an alcohol with three hydroxyl functional groups.

1,2,3 Propanetriol
($CH_2OHCHOHCH_2OH$)



c. **Comparison of Alcohols and Inorganic Hydroxides**. Since alcohols do not ionize, their reactions are much slower than inorganic reactions. If an alcohol solution is tested for $OH^-$ ions with litmus paper, no change in the color of the paper will be observed as there is when a sodium hydroxide solution is tested. Alcohols react with acid to form a new compound and water. Reactions between organic acids and hydroxides yield a salt plus water. The compound formed when alcohols and organic acids react is called an **ester**.

(1) Inorganic reaction.

$$NaOH + CH_3COOH \longrightarrow Na^+CH_3COO^- + H_2O$$

Sodium hydroxide + Acetic acid ——> Sodium acetate + Water
(Base) (salt)

(2) Organic reaction.

$$CH_3OH + CH_3COOH \longrightarrow CH_3OCOCH_3 + H_2O$$

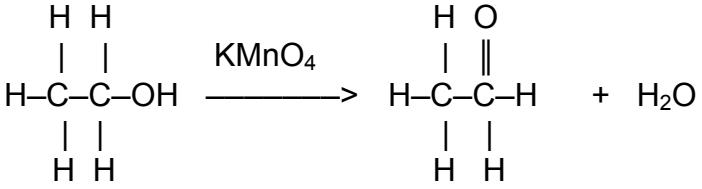
Methanol + Acetic acid ——> Methyl acetate + Water
(Alcohol) (Ester)

d. **Reactions**. The hydroxyl group or bond which attaches the hydroxyl group to a carbon is the site for many of the reactions of alcohols.

(1) Combustion. Alcohols are flammable organic solvents which can be easily ignited near an open flame. The resulting products are carbon dioxide, water, and energy.

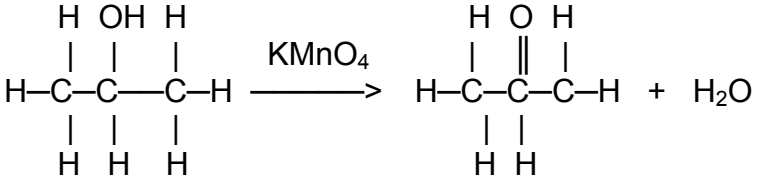
(2) Oxidation. Inorganic oxidation involves the loss of electrons from an atom(s) during a chemical reaction. Organic oxidation differs in that there is a gain of oxygen or a loss of hydrogen with their accompanying electrons. All oxidation reactions require an oxidizing agent for the reaction to proceed. Permanganate ($KMnO_4$) and dichromate ($K_2Cr_2O_7$) are examples of oxidizing agents.

(a) Primary alcohols, when oxidized, will form water and an organic compound called an aldehyde (suffix –al).




Ethanol Ethan**al**

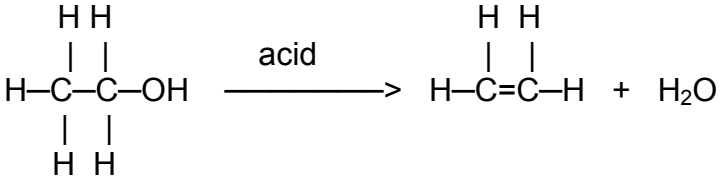
(b) Secondary alcohols, when oxidized, will form water and an organic compound called a ketone (suffix –one).




2–Propanol 2–Propan**one**

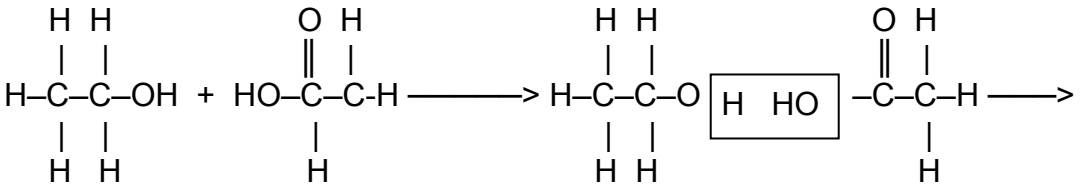
(c) Tertiary alcohols will not oxidize under normal conditions because the carbon, attached to the hydroxyl group, does not have a free hydrogen which can be removed.

(3) Dehydration. This reaction involves the removal of the hydroxyl group and must occur under acidic conditions. The resulting products are water and an alkene.

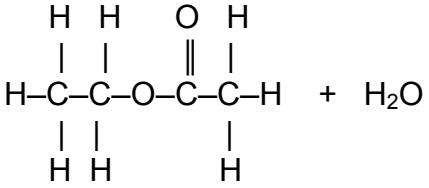



Ethanol Ethene

(4) Esterification. When alcohols are reacted with organic acids, the resulting products are water and an organic compound called an ester (suffix –ate).

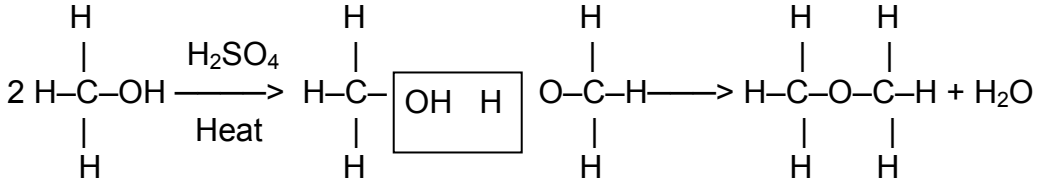



Ethanol Ethanoic acid




Ethyl ethanoate

(5) Ether formation. This reaction is a kind of dehydration in which excess alcohol in a sulfuric acid solution yields ether and water.

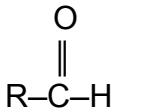



Methanol
(2 represents an excess
of methanol is needed)

Dimethyl
Ether

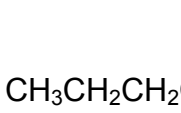
## 5-5. ALDEHYDES

a. **Aldehydes.** Aldehydes may be regarded as hydrocarbon derivatives in which two of the hydrogen (H) atoms attached to a carbon (C) at the end of a hydrocarbon chain have been replaced by an oxygen (O) atom. They contain the characteristic functional group shown below. The carbon double bonded to the oxygen is called the carbonyl functional group. This functional group is found in both aldehydes and ketones.




Where: O=C is the carbonyl group.
It can also be written –CHO.

b. **IUPAC Nomenclature.** Aldehydes are named by first identifying the longest continuous hydrocarbon chain that contains the carbonyl group. Then the –e ending of the hydrocarbon is replaced by the suffix –al. The common names of aldehydes are accomplished by using the name of the organic acid that contains the same number of carbons, dropping the –ic acid and adding the suffix -aldehyde. Thus methanal is called formaldehyde, ethanol is acetaldehyde and propanol is called propionaldehyde.



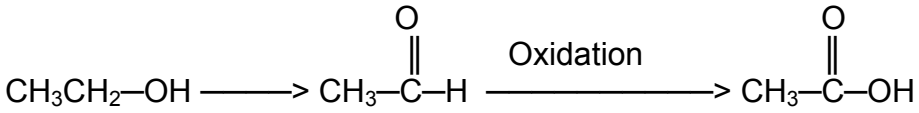

Butanal (Butyraldehyde)

c. **Methanal (Formaldehyde)**. Formaldehyde is a gas with a characteristic odor. In a solution known as formalin, it has been used as a popular tissue preservative. Formaldehyde exhibits the phenomenon of polymerization; that is, molecules of formaldehyde tend to unite with each other to form new molecules, each containing three formaldehyde molecules. This compound is known as paraformaldehyde $(HCHO)_3$ and is said to be a polymer of formaldehyde.

d. **Ethanal (Acetaldehyde)**. This substance polymerizes to form paraldehyde $(CH_3CHO)_3$, which has been used in medicine as a sleep-producing drug or as a sedative. Another hypnotic made from acetaldehyde is chloral, $CCl_3CHO$. Chloral combines with water to form a crystalline solid known as chloral hydrate, which has been used in bacteriological media to prevent the swarming of Proteus organisms.

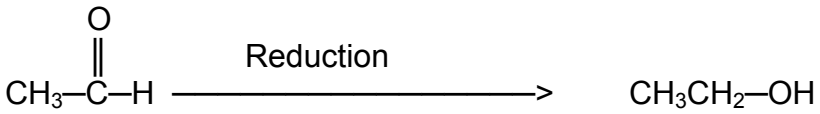
e. **Reactions**.

(1) As discussed earlier, primary alcohols can be oxidized to aldehydes.




Ethanol — Ethanal (Acetaldehyde) — Ethanoic acid or Acetic acid

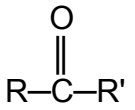
(2) In referring to the reactions of alcohols, it is observed that the oxidation of a primary alcohol produces an aldehyde. One also sees a reverse reaction, the reduction of an aldehyde to produce an alcohol. It is also understood that the continued oxidation of a primary alcohol can result in the formation of an organic acid.




Acetaldehyde — Ethanol

## 5-6. KETONES

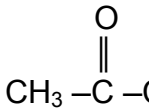
a. **Ketones**. Ketones are formed by the oxidation of a secondary alcohol. If a ketone is reduced, it again forms the same secondary alcohol from which it was formed. Their characteristic structure is:




Where: R and R' can be the same or different hydrocarbon groups.

b. **Neutral Compounds**. Ketones are neutral compounds being neither acids nor bases.

c. **Functional Group**. The ketone functional group appears in the structure of many complex drugs, such as steroid compounds and vitamins. Simple ketones, with the exception of acetone, are seldom used in medicine.

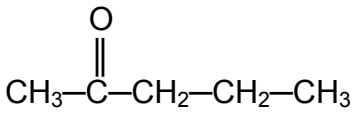


2–propanone (Acetone)

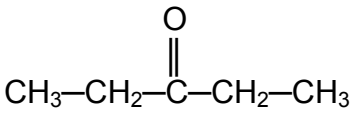
d. **2–Propanone (Acetone)**. Acetone is widely used as a solvent in clinical laboratory work. It is found in trace amounts in normal blood and urine. In uncontrolled cases of diabetes mellitus, large amounts are present in blood, urine, and also in the patient's breath.

e. **Acetoacetic Acid ($CH_3COCH_2COOH$)**. This compound, which contains both a ketone and an acid group, also occurs in the blood and urine of diabetic patients.

f. **IUPAC Nomenclature**. Ketones are named by first identifying the longest continuous hydrocarbon chain that contains the carbonyl group. Then the –e ending is changed to the suffix –one. There are several locations in a chain where the carbonyl group could be placed. Its position is designated by the lowest possible number. The ketones are also designated by naming the two aliphatic groups and then ending the name ketone.

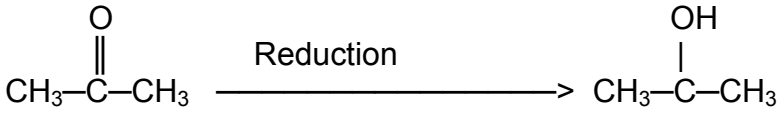


2–Pentanone



3–Pentanone

g. **Reactions**. Ketones are very resistant to further oxidation and for all practical purposes, it can be stated that they do not undergo further oxidation. Ketones are similar to aldehydes in their boiling points, which are lower than those of corresponding alcohols and organic acids. The equation for the reduction of a ketone is as follows:



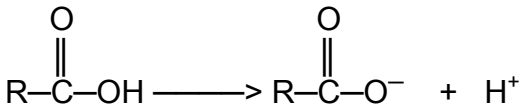

Acetone 2–Propanol

## 5-7. ORGANIC ACIDS

a. **Organic Acids**. Organic acids are a class of organic compounds characterized by the carboxyl group, whose name comes from the words carbonyl and hydroxyl, its two functional groups. The carboxyl group is often written ─COOH.

Where: O=C is the carbonyl group; -OH the hydroxyl group. It can also be written as -COOH.

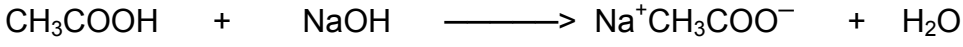
(1) Organic acids are acids because they ionize in solution to give a carboxylate ion and a proton.



carboxylate ion

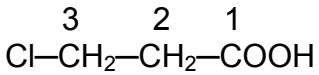
proton

(2) They resemble inorganic acids in that they react with inorganic bases to produce organic salts and water. Organic acids are among the weakest acids; organic bases are among the weakest bases.

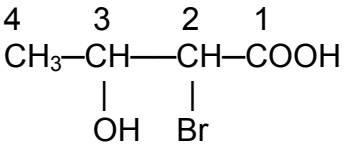


Acetic acid + Sodium Hydroxide ——> Sodium acetate + Water
(Organic acid) + (Inorganic base) ——> (Organic salt)

b. **IUPAC Nomenclature**. Organic acids are named by identifying the longest continuous hydrocarbon chain containing the carboxyl group. Then the ─e ending of the parent alkane is replaced by the suffix ─oic acid. The carboxyl group is numbered as carbon 1. Each substituent on the chain is identified by its name and a number indicating its position on the chain.



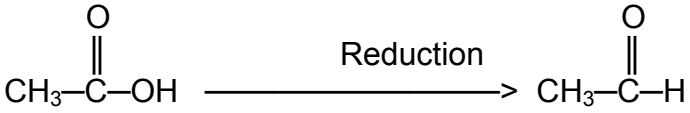
3─Chloropropanoic acid



2─Bromo─3─hydroxybutanoic acid

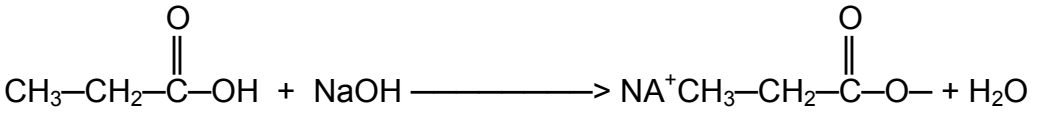
c. **Reactions**. Organic acids are formed by the oxidation of an aldehyde. They can thus be reduced to form aldehydes. As stated before organic acids, being weak acids, react with bases. The reaction of an organic acid with a strong base such as potassium hydroxide results in the formation of the potassium salt of that acid and water. If an organic acid reacts with an organic base such as amine, the resulting product is called an amide. This is the same reaction that takes place to covalently bond amino acids to produce protein molecules. Organic acids also react with alcohols to form esters. The equations for the reduction of an organic acid and the reaction with bases are as follows:

(1) Reduction of an organic acid



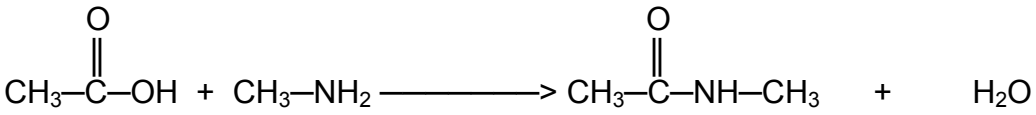

Acetic acid Acetaldehyde

(2) Reaction of organic acid with a strong base




Propanoic acid Sodium propionate + Water

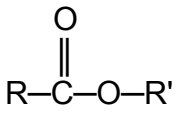
(3) Reaction of organic acids with amines




Acetic acid Methylamine N—methylacetamide + Water

## 5-8. ESTERS

a. **Esters**. Esters are the result of the chemical combination of an organic acid in which the ─OH of the carboxyl group has been replaced by the ─OR from an alcohol. Esters contain a carbonyl group and an ether link to the carbonyl carbon. They contain the functional group

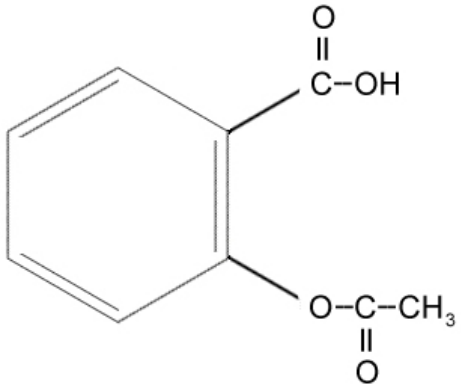


where C=O is from the acyl group from the acid; O─R' is the alkyl or aryl group from the alcohol. The abbreviated formula for a carboxylate ester is RCOOR. The R groups can be short chains or long chains, aliphatic (alkyl) or aromatic (aryl), saturated or unsaturated.

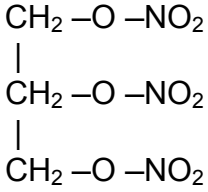
b. **Characteristics.** The simplest esters are liquids and have fragrant odors. An example is ethyl ethanoate (Ethyl acetate) $CH_3–CH_2–OOC–CH_3$), which has the odor of pineapple. Esters cannot form hydrogen bonds [a weak electrostatic attraction between one electronegative atom (O or N) and a hydrogen atom covalently linked to a second electronegative atom (O)] between themselves; consequently, they have boiling points similar to alkanes of similar molecular weight. They can form hydrogen bonds with water; therefore, esters that contain less than five carbon atoms are soluble.

c. **Functional Group**. The ester functional group is found in many complex drug molecules which one would study in pharmacology. Some examples are shown below.

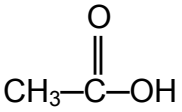
Acetylsalicylic Acid (Aspirin) –an analgesic



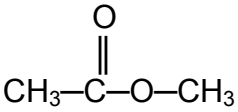
Nitroglycerin –a cardiac drug



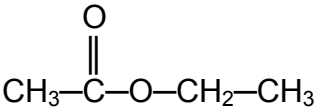
d. **IUPAC Nomenclature**. Esters are named as derivatives of organic acids (names contain two words). The first word comes from the alkyl or aryl group (alcohol) and the second from the acyl group (acid) which has the –ic suffix changed to –ate. The example uses acetic acid as the parent compound.



Acetic acid



Methyl acetate

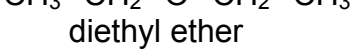


Ethyl acetate

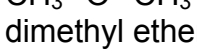
e. **Reactions**. The formation of esters has been discussed in the previous sections on alcohols and organic acids. Esters undergo hydrolysis to form the organic acid and the alcohol from which the ester was formed.

## 5-9. ETHERS

a. **Carbon Chains or Rings**. Ethers are compounds in which both the hydrogens of water are replaced by carbon chains or rings. They are organic compounds that have R─O─R as the functional group. Some examples of ethers are:



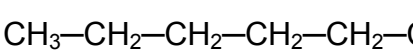
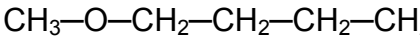
diethyl ether



dimethyl ether

divinyl ether

b. **Polar**. Ether molecules are slightly polar, but cannot form hydrogen bonds with each other since they do not have a hydrogen atom attached directly to an oxygen atom. Therefore, they have about the same boiling points and melting points as alkanes of similar molecular weights (M.W.).

| | M.W. | Boiling Point |
|---|---|---|
|  | 100 | 98°C |
| Heptane (Alkane) | | |
|  | 102 | 100°C |
| Methyl pentyl ether | | |

c. **Soluble in Water**. Since ether molecules are slightly polar and have an oxygen atom in their structure, they can form hydrogen bonds with water. This property accounts for the fact that ethers are slightly soluble in water.

d. **IUPAC Nomenclature**. Ethers are easy to name. They are designated by naming the two aliphatic groups and adding the word ether. When both R groups are the same, the ether is referred to as being symmetric or simple. Symmetric ethers are named by using the prefix di–.

| | |
|---|---|
| $CH_3–CH_3–O–CH_3$ | $CH_3–CH_2–O–CH_2–CH_3$ |
| (Ethyl methyl ether) | (Diethyl ether) |

Sometimes the prefix di–is dropped and the compound, such as dimethyl ether, is simply called ethyl ether. Ethers may also be named as an alkoxy derivative. For example, methyl ethyl ether is named methoxyethane.

e. **Reactions.** As discussed previously, ethers are formed by the dehydration of alcohols. Chemically, ethers are inert except for oxidation reactions. Ethers are very unstable compounds in the presence of peroxides and very subject to combustion. Medicinally, ethers have been used as general anesthetics.

## 5-10. AMINES

a. **Number of Carbon Groups**. Amines are organic derivatives of ammonia ($NH_3$). They are called primary, secondary, or tertiary, depending on the number of R groups attached to the nitrogen.

| | | | |
|---|---|---|---|
| H<br>\|<br>H–N–H | H<br>\|<br>R–N–H | R<br>\|<br>R–N–H | R<br>\|<br>R–N–R |
| Ammonia | Primary Amine | Secondary Amine | Tertiary Amine |

(1) The terms primary, secondary, and tertiary are used quite differently than with alcohols. In alcohols, these terms referred to the number of carbon groups attached to the carbon.

(2) In amines, they refer to the number of carbon groups attached to the amine nitrogen. The carbon group can be aliphatic, aromatic, or both.

b. **Volatile Liquids**. The low molecular weight amines are all volatile liquids; and, those having up to five carbons are soluble in water. The element nitrogen is in the same period of the periodic table as oxygen and has some similar properties, the most significant being the ability to form hydrogen bonds. The formation of hydrogen bonds between amines and between amines and water accounts for their higher boiling points (than alkanes) and increased water solubility.

c. **Basic Changes**. Since amines are derivatives of ammonia, they are bases as defined by the Bronsted–Lowry theory. The nitrogen of the amine can accept a proton to form a substituted ammonium ion.

$$CH_3–CH_2–NH_2 + H^+ \longrightarrow CH_3–CH_2–NH_3^+$$

(1) Amines will thus react with inorganic acids to form salts. Amines react with organic acids to form amides, a class of organic compounds, discussed in paragraph 5–11.

$$CH_3–NH_2 + HCl \longrightarrow CH_3–NH_3^+Cl^-$$

(2) The reaction in the example above results in a hydrochloride salt of the amine and is very important in medicine. Many drugs contain an amine functional group; and, if they contain a lot of carbon atoms, they are not very soluble in water. The salts formed from amines, however, are very soluble in water. Therefore, if you wish to use a water solution of an amine drug, which is insoluble, you can make it soluble by forming the salt of the amine.

d. **IUPAC Nomenclature**. Aliphatic amines are named by first identifying the alkyl groups bonded to the amine nitrogen and attaching the word –amine. The name of the aliphatic groups is followed by the word –amine and is written as one word. The prefixes di– and tri– prefixes are used to indicate more than one aliphatic group of the same kind.

| | | |
|---|---|---|
| $CH_3–\underset{\displaystyle H}{\underset{\vert}{N}}–H$ | $CH_3–\underset{\displaystyle H}{\underset{\vert}{N}}–CH_3$ | $CH_3CH_2–\underset{\displaystyle CH_3}{\underset{\vert}{N}}–CH_3$ |
| Methylamine | Dimethylamine | Dimethylethylamine |

e. **Reactions**. One of the more common methods of preparing an amine is to displace a halogen in a hydrocarbon with ammonia or an amine nitrogen. Then, treat the salt with a base to release the free amine.

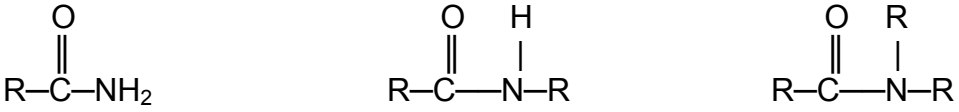
$$\underset{\displaystyle H}{\underset{\vert}{H}}–\underset{\displaystyle H}{\underset{\vert}{N}}–H \;+\; R–I \longrightarrow R–NH_3^+\; I^-$$

Ammonia Halide Ammonium salt

$$R–NH_3^+I^- + NaOH \longrightarrow \text{amine } (R–NH_2) + NaI + H_2O$$

## 5-11. AMIDES

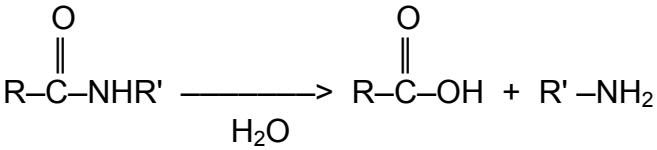
a. **Derivatives of Organic Acids**. Amides are ammonia or amine derivatives of organic acids. They may be simple, mono-substituted, or disubstituted.



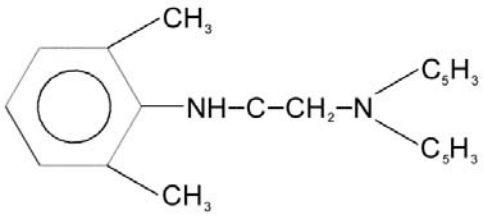
Simple amide | Monosubstituted amide | Disubstituted amide

b. **Hydrogen Bonding**. Amides, because of the hydrogen attached to the nitrogen atom, can form hydrogen bonds between themselves. They have higher boiling and melting points than corresponding alkanes. Since they can also form hydrogen bonds with water, amides containing up to five carbon atoms are soluble in water.

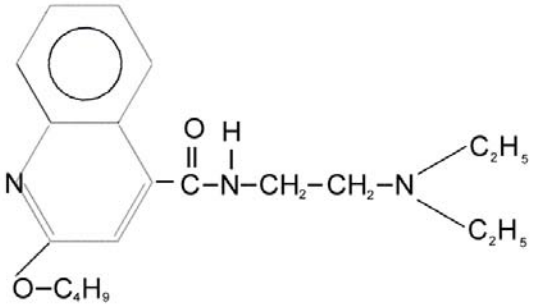
c. **Hydrolysis**. Amides are neutral in pH and undergo the hydrolysis reaction. For amides, hydrolysis is the splitting of the compound with the incorporation of water to form a carboxylic acid and an amine.



d. **Drug Molecules**. Some examples of drug molecules containing the amide functional group are shown below.

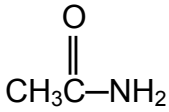


Lidocaine (Xylocaine®) Local anesthetic

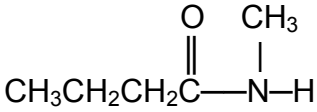


Dibucaine (Nupercaine®) Local anesthetic

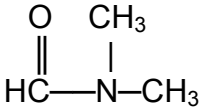
e. **IUPAC Nomenclature**. Amides are named by dropping the –ic or –oic ending from the parent acid and adding the suffix –amide. Any substituents on the amine nitrogen are named as prefixes preceded by N–or N,N–.



Acetamide



N–Methylbutanamide



N,N–Dimethylformamide

f. **Reactions**. Methods of preparing an amide involve dehydrating ammonium salts of organic acids or reacting ammonia or an amine with either an ester or an organic acid anhydride.

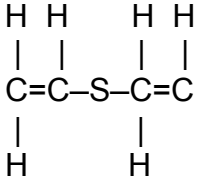
## 5-12. THIOLS (MERCAPTANS)

a. **Derivatives of Sulfides**. Alcohols and ethers are organic derivatives of water; so thiols and thioethers are organic derivatives of hydrogen sulfides ($H_2S$). Thiols are compounds with the general formula R–S–H. Thioethers are compounds with the general formula R–S–R and are usually called sulfides.

b. **IUPAC Nomenclature**. Thiols are named by adding the suffix –thiol to the name of the parent hydrocarbon. Note that the –e ending is not deleted. The <u>common names of thiols</u> are formed by first naming the alkyl group and then adding the name mercaptan.



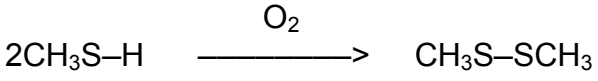
Methanethiol
(Methyl mercaptan)



Divinyl sulfide

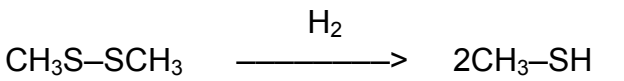
c. **Reactions**.

(1) Thiols are easily oxidized to disulfide:



Methanethiol Methyl disulfide

(2) Disulfides are easily reduced to thiols. Although many reducing substances are available, hydrogen works well:




Methyl disulfide Methanethiol

**Continue with Exercises**

**EXERCISES, LESSON 5**

**INSTRUCTIONS:** Answer the following exercises by marking the lettered response that best answers the question, by completing the incomplete statement, or by writing the answer in the space provided at the end of the question.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Which one element provides the significance to create another division of chemistry, that being organic chemistry?

   a. Oxygen.

   b. Sodium.

   c. Carbon.

   d. Hydrogen.

2. How many free electrons does the carbon atom have in its outermost shell?

   a. 1.

   b. 2.

   c. 3.

   d. 4.

3. Carbon can form bonds between itself in the shape of long chains, branched chains, or _____ structure.

   a. Square.

   b. Ring.

   c. Diamond.

   d. Parallelogram.

4. A covalent bond is defined as:

   a. Carbon forming bonds by sharing its electrons with other atoms.

   b. Carbon forming bonds by sharing its protons with other atoms.

   c. Carbon bonding or sharing its neutrons with other atoms.

   d. Zinc forming bonds by sharing its electrons with other atoms.

5. Which structural formula is the molecular formula for ethane?

   a. $CH_3CH_2CH_3$.

   b. $CH_3CH_3$.

   c. $CH_3CH_2–OH$

   d. $CH_3–O–CH_3$

6. Carbon combines with divalent and trivalent elements to form:

   a. Univalent atoms, and aromatic and trivalent rings of carbon atoms.

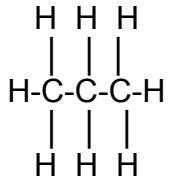
   b. Rings of sodium atoms and chains of carbon atoms.

   c. Double and triple bonds that are represented by double and triple dashes.

   d. Nicotinic atoms, divalent and trivalent bonds, rings of carbon atoms, and chains of carbon atoms.

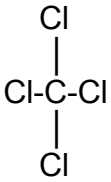
7. Which is a simple structural formula of carbon where a single unit of carbon unites with an element that is univalent to form only single bonds?

a.



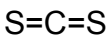
Propane ($C_3H_8$)

b.

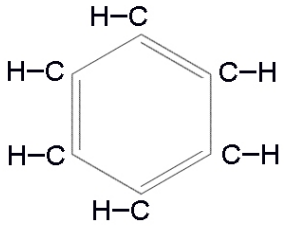


Carbon tetrachloride ($CCl_4$)

c.

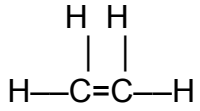


Carbon disulfide ($CH_2O$)



Benzene ($C_6H_6$)

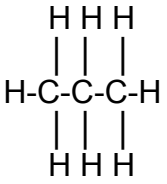
d.



Ethene ($C_2H_4$)

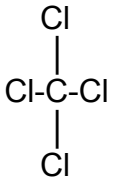
8. Which structural formula is representative of the chains of carbon atoms?

a.



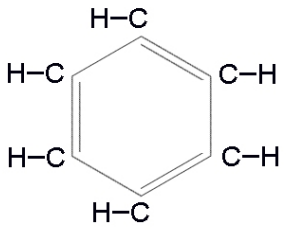
Propane ($C_3H_8$)

b.



Carbon tetrachloride ($CCl_4$)

c.




Benzene ($C_6H_6$)

d.

S=C=S

Carbon disulfide ($CS_2$)

9. Which elements below will form four single bonds to carbon? These bonds represent the sharing of one pair of electrons between the carbon atoms and the non-carbon atoms?

a. Halogens, such as Cl and F.

b. Nonmetals, such as B and S.

c. Heavy metals, such as Fe and Tc.

d. Halogens, such as Ge and Na.

10. Which statement best describes the difference between rings and chains?

    a. The H atoms in a ring share electrons with each other in a closed–circuit arrangement.

    b. The O atoms in a ring share electrons with each other in a closed–circuit arrangement.

    c. The C atoms in a ring share electrons with each other in a closed–circuit arrangement.

    d. A single unit of C unites with an element.

11. Rings of carbon atoms can result when:

    a. Branches are formed.

    b. An elongated form evolves.

    c. Carbon atoms unite with each other.

    d. A saturated organic compound is formed.

12. Organic compounds having the combining capacities of all the carbons satisfied are known as:

    a. Saturated compounds.

    b. Isomers.

    c. Abbreviated bonds.

    d. Other carbons or non-carbon atom structures.

13. Propanal is what type of organic compound and why? It is:

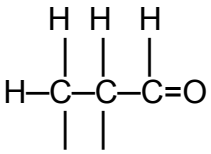
    a. An organic catalyst because it is reluctant to participate in chemical reactions.

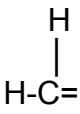
    b. Unsaturated because not all of the carbon atoms are joined by a single bond.

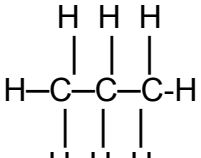
    c. Unsaturated because the combining capacities of all the carbons are satisfied.

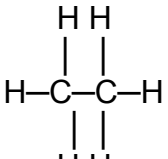
    d. A saturated organic compound because all the carbon to carbon bonds are single bonds.

14. Which formulas are saturated compounds?

    1.  Propanal ($C_3H_6O$)

    2.  Ethene (Ethylene ($C_2H_4$))

    3.  Propane ($C_3H_8$)

    4.  Ethane ($CH_3CH_3$)

    a. 1 and 3.

    b. 1, 2, 3.

    c. 2, 3, 4.

    d. 1, 3, 4.

15. Unsaturated compounds:

    a. Contain at least one carbon atom joined by a double or triple bond.

    b. Contain at least two carbon atoms joined by a double or triple bond.

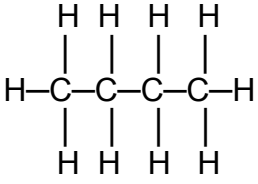
    c. May form new compounds, when bound with other atoms.

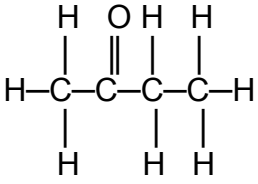
    d. Are more chemically active than saturated compounds because double and triple bonds are less stable than single bonds.

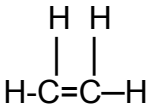
    e. a and b.

    f. b, c, and d.

16. Which structural formula demonstrates an unsaturated compound?

    a. 
    Butane ($CH_3(CH_2)_2CH_3$)

    b. 
    2–Butanone ($CH_3–C–CH_2CH_3$)

    c. 
    Ethene (Ethylene ($C_2H_4$))

17. Which basic portion of an organic structure, involved in chemical reactions, is defined as follows?

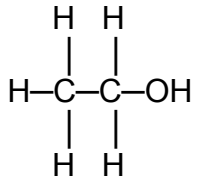
    a. Division or organic compounds.

    b. Saturated compound.

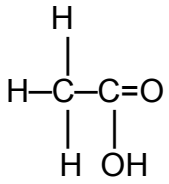
    c. Functional group.

    d. Battalion group.

18. This group may be a specific type of bond, an atom that has replaced hydrogen, or a radical. To which basic structures do these bonding effects belong? The -COOH can substitute for the -OH.



Ethanol ($C_2H_5OH$)



Ethanoic acid ($CH_3COOH$)

a. Division or organic compounds.

b. Saturated compound.

c. Functional group.

d. Company group.

19. The divisions of organic compounds are composed of:

a. Carbocyclic, alphanumeric, aliphatic, and heterocyclic compounds.

b. Aliphatic, carbocyclic, alkane, and heterocyclic compounds.

c. Carbocyclic, aromatic, aliphatic, and heterocyclic compounds.

d. Alkynes, carbocyclic, aliphatic, and heterocyclic compounds.

20. An aliphatic compound is defined as:

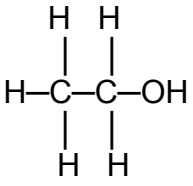
a. An organic compound in which the molecules are composed of open or branched chains of carbon atoms to which atoms or radicals are attached.

b. An organic compound which is composed of rings of carbon atoms.

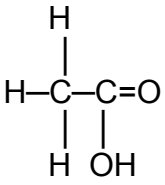
c. An organic compound which has at least two carbon atoms joined only by a double or triple bond.

d. A compound in which all of the combining capacities of all the elements are satisfied.

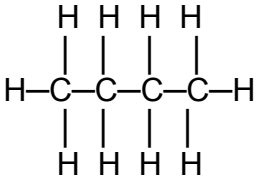
21. Which compounds are aliphatic?

1. 

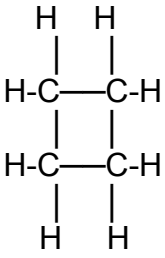
   Ethanol ($C_2H_5OH$)n

2. 

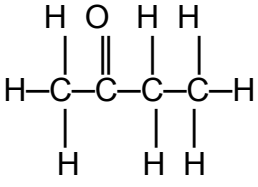
   Ethanoic acid ($CH_3COOH$)

3. 

   Butane ($CH_3(CH_2)_2CH_3$)

4. 

   Cyclobutane ($CH_2CH_2CH_2CH_2$)

5. 

   2–Butanone ($CH_3–C–CH_2CH_3$)

a. 1, 2, 5.

b. 2, 3, 4.

c. 2, 5.

d. 3, 5.

22. Which ring compounds have some other element in addition to carbon in the ring?

    a. Aliphatic.

    b. Carbocyclic.

    c. Heterocyclic.

    d. Aromatic.

23. Hydrocarbon compounds contain which grouping of compounds?

    a. Alkenes, alkanes, amines, alkynes, and allspice.

    b. Alkynes, alkenes, alkanes, and butane.

    c. Alkanes, alkynes, and alkalines.

    d. Alkanes, alkynes, and alkenes.

24. Define the alkane hydrocarbons.

    a. Compounds which contain at least one carbon to carbon double bond.

    b. Saturated aliphatic compounds which may be considered to be derivatives of methane, the simplest member of the group.

    c. Unsaturated hydrocarbons which contain at least one carbon to carbon triple bond.

    d. A member of the hydroxyl group which has the second carbon forming two carbon to carbon bonds, whereas the first carbon forms only one.

25. Which hydrocarbons have unsaturated carbons that contain at least one carbon to carbon triple bond?

    a. Alkenes.

    b. Alkanes.

    c. Alkynes.

    d. Aromatic.

26. What is the first step in naming an alkane as per the IUPAC?

    a. Identify the shortest broken chain of carbon atoms.

    b. Select the first broken chain in the carbon atoms.

    c. Identify the longest unbroken chain of carbon atoms.

    d. Look for the seven carbon long straight chain compound that has only carbon to carbon single bonds.

27. If a number prefix is used to denote the name of an alkane, based on the number of carbons in the chain, which is correct?

    a. 1 carbon is meth–, 2 carbons is prop–, 3 is eth–, 4 is but–, 5 is pent–, 6 is hex–, 7 is hept–, 8 is oct–, 9 is non–, and 10 carbons is dec–.

    b. 1 carbon is meth–, 2 carbons is eth–, 3 is prop–, 4 is but–, 5 is pent–, 6 is hex–, 7 is oct–, 8 is hect–, 9 is non–, and 10 carbons is dec–.

    c. 1 carbon is meth–, 2 carbons is eth–, 3 is prop–, 4 is pent–, 5 is but–, 6 is hex–, 7 is hept–, 8 is oct–, 9 is non–, and 10 carbons is dec–.

    d. 1 carbon is meth–, 2 carbons is eth–, 3 is prop–, 4 is but–, 5 is pent–, 6 is hex–, 7 is hept–, 8 is oct–, 9 is non–, and 10 carbons is dec–.

28. To indicate that a compound is an alkane, which suffix is added to the prefix?

    a. -ane.

    b. -ene.

    c. -yne.

    d. -one.

29. Why do alkanes have limited reactivity?

    a. They are heavy.

    b. The stability of the saturated carbon to carbon bonds causes this.

    c. Their seven carbon long straight chain prevents this.

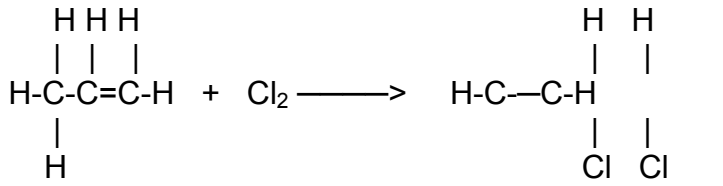
    d. They are not vigorous moving atoms. They are slow to combust.

30. Alkanes are popular combustible fuels and readily react with oxygen. What do they form and what equation represents this combustible reaction?

    a. Carbon dioxide, water, and energy are formed.

    $CH_4 + 2\ O_2 \longrightarrow CO_3 + 2\ H_2O$ + Heat Energy

    b. Propane and 1,2 Dichloroethane are formed.



    Propene 1,2 Dichloroethane

    c. Carbon dioxide, water, and energy are formed.

    $CH_5 + 2\ O_2 \longrightarrow CO_2 + 2\ H_2O$ + Energy

    d. Carbon dioxide, water, and energy are formed.

    $CH_4 + 2\ O_2 \longrightarrow CO_2 + 2\ H_2O$ + Energy

31. What type of system is used to identify which carbon is attached to the double bond with alkene compounds?

    a. Numbering.

    b. Letter.

    c. Alphanumeric.

    d. Greek.

32. With alkene compounds, what is an addition reaction?

    a. When alkenes occur at the single bond, it is broken and a pair of electrons are available to form bonds with halogens, hydrogen, or hydroxyl group ($OH^-$).

    b. The double bond is broken and a pair of electrons are available to form bonds with halogens, hydrogen, or hydroxyl group ($OH^-$).

    c. When they occur at the double bond, the bond is broken and a pair of protons are available to form bonds with halogens, hydrogen, or hydroxyl group ($OH^-$).

    d. The halogens are added to an alkene without the formation of an acid.

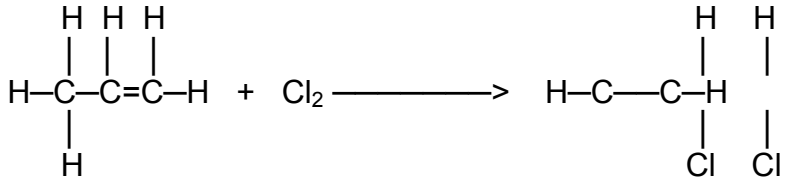
33. What is halogenation?

    a. Halogens are added to an alkene without the formation of an acid.

    b. The resulting product is an alkane with a halogen atom attached to each carbon, which shared a double bond.

    c. a and b.

    d. None of the above but:



Propene 1,2 Dichloroethane

34. How does hydrogenation differ from halogenation?

    a. The carbon is the reactant and is subtracted from the double bond and a catalyst.

    b. Hydrogen is the reactant added to the double bond. A catalyst is required for the resulting product, alkane.

35. What is the resulting product for this statement? One or more of the hydrogens of the hydrocarbons is replaced by a hydroxyl to form a/an:

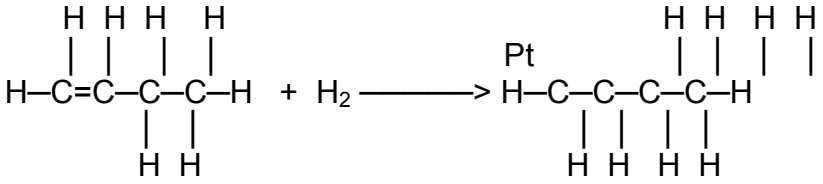
    a. Propene.

    b. Pentane.

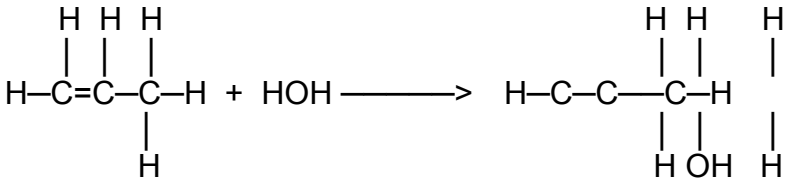
    c. Propanol.

    d. Alcohol.

36. Which reactions do these two equations represent?




1–Butene Butane



1–Propene Water 2–Propanol

    a. Halogenation and addition reaction.

    b. Heterocyclic and halogenation.

    c. Hydrogenation and hydration.

    d. Hydration and halogenation.

37. Which suffix is used to indicate that a compound is an alcohol and what is replaced by the hydroxyl ion?

    a. -ene; oxygen.

    b. -yne; hydrogen

    c. -ol; hydrogen.

    d. -ane; water.

38. Which symbol is representative of the hydroxyl ion?

    a. -NCOOH.

    b. $-CH_2$.

    c. -COOH.

    d. $-OH^-$.

39. Based on the number of alcohol groups, how many types of alcohol are there?

    a. 1.

    b. 2.

    c. 3.

    d. 4.

40. Which statement is correct for secondary classification of alcohol?

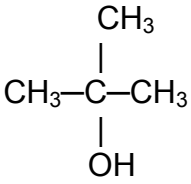
    a. The hydroxyl ion is attached to the terminal carbon; a carbon which shares a bond with only one other carbon.

    b. The carbon atom, to which the hydroxyl group is attached, is bonded to two other carbon atoms.

    c. The hydroxyl group is attached to a carbon which also forms bonds with three other carbons.

    d. None of the above.

41. This structural formula represents which classification of monohydric of alcohol?



2–Methyl–2–Propanol

a. Primary.

b. Secondary.

c. Tertiary.

42. The suffix –triol indicates that the compound is an alcohol with three of the same functional groups. This is indicative of which type of alcohol?

a. Monohydric.

b. Dihydric.

c. Trihydric.

43. When oxidized, what will a primary alcohol form?

a. Water and an organic compound called an aldehyde.

b. Water and an organic compound called ketone.

c. They will not oxidize.

d. a and b.

44. What is esterification?

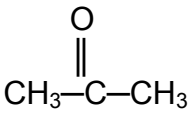
    a. This reaction involves the removal of the hydroxyl group and must occur under acidic conditions. The resulting products are water and an alkene.

    b. When alcohols are reacted with organic acids, the resulting products are water and an organic compound called an ester.

    c. This reaction is a kind of dehydration in which excess alcohol in a sulfuric acid solution yields ether and water.

    d. It is the same as dehydration.

45. Select the category of organic compounds to which the following substance belongs:



    a. Organic acid.

    b. Aldehyde.

    c. Ketone.

    d. Amine.

46. Select the type(s) of reaction(s) ketones will undergo.

    a. Oxidation.

    b. Reduction.

    c. Hydrolysis.

    d. Both a and b.

47. Given the structure $CH_3COOH$, determine its IUPAC nomenclature.

    a. Ethanoic acid.

    b. Methanoic acid.

    c. Formic acid.

    d. Pentanoic acid.

48. Given the structure $CH_3OCH_3$, determine its IUPAC nomenclature.

    a. Acetone.

    b. Acetaldehyde.

    c. Dimethyl ketone.

    d. Dimethyl ether.

49. Amines will react with a halogen to form:

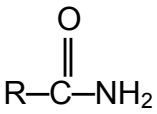
    a. Amides.

    b. Ketones.

    c. Salts.

    d. Aldehydes.

50. Select the category of organic compounds to which the following substance belongs:



a. Organic acid.

b. Aldehyde.

c. Ketone.

d. Amide.

51. Thiols and thioethers are organic derivatives of what compound?

a. Ammonium salt.

b. Hydrogen dioxide.

c. Hydrogen sulfide.

d. Ethyl acetate.

52. The **common** name of thiol is formed by naming the _____ group and then the name _____.

a. Alkyl; ohcaptan.

b. Alkyl; mercaptan.

c. Amide; mercaptan.

d. Ether; yocaptan.

**Check Your Answers on Next Page**

**SOLUTIONS TO EXERCISES, LESSON 5**

1. c (para 5-1)
2. d (para 5-2)
3. b (para 5-2d)
4. a (para 5-2)
5. b (para 5-2a)
6. c (para 5-2b)
7. b (para 5-2b)
8. a (para 5-2c)
9. a (para 5-2b)
10. c (para 5-2d)
11. c (para 5-2d)
12. a (para 5-2e)
13. d (para 5-2e)
14. d (para 5-2e)
15. f (para 5-2f)
16. c (para 5-2f)
17. c (para 5-2g)
18. c (para 5-2g)
19. c (para 5-2h)
20. a (para 5-2h(1))
21. d (para 5-2h(1))
22. c (para 5-2h(3))

23. d (para 5-3)

24. b (para 5-3a)

25. c (para 5-3d)

26. c (para 5-3a(1)(a))

27. d (para 5-3a(1)(a))

28. a (para 5-3a(1)(b))

29. b (para 5-3a(2)(a))

30. d (para 5-3a(2)(a))

31. a (para 5-3c(1)(a))

32. b (para 5-3c(2))

33. c (para 5-3c(2)(a))

34. b (para 5-3c(2)(b))

35. d (para 5-3c(2)(c))

36. c (para 5-3c(2)(b),(c))

37. c (para 5-4, 5-4a)

38. d (para 5-4)

39. c (para 5-4b)

40. b (para 5-4b(1)(b)

41. c (para 5–4b(1)(c))

42. c (para 5-4b(3))

43. c (para 5-4c)

44. b (para 5-4d(4))

45. c (para 5-6c)

46. b (para 5-6g)

47. a (para 5-7b)

48. d (para 5-9d)

49. c (para 5-10c)

50. c (para 5-11c)

51. c (para 5-12a,c)

52. b (para 5-12b)

**End of Lesson 5**