Outline

At the end of the session, the student must be able to learn:

- **I. Laboratory Hazards**
  - A. Occupational Hazard
    - 1. Fire Hazards
    - 2. Chemical Hazards
      - **i.** MSDS Diamond
    - 3. Electrical Hazards
    - 4. Sharp Hazard
  - B. Physical Hazard
    - 1. Ergonomic Hazard
    - 2. Vibration Hazard
    - 3. Noise Hazard
  - C. Other Hazards
    - 1. Biological Hazard
    - 2. Radiation Hazard
- **II. Biosafety Level**
  - A. Safety Precautions
    - 1. OSHA Standards
      - i. Handwashing Procedure
- **III. Laboratory Waste Management**
  - A. Segregation
    - 1. Biodegradable
    - 2. Non-Biodegradable
    - 3. Hazardous Waste
      - i. Special waste
      - ii. Biological Waste
      - iii. Chemical Waste
      - iv. Radioactive Waste
  - B. Waste Disposal Standard
    - 1. Blood Containing Waste
      - i. Standard Waste Protocol (PHIL)
  - C. Disposal
    - 1. Flushing Down the Drain to the Sewer System
    - 2. Incineration
    - 3. Landfill Burial
    - 4. Recycling
- **IV. Occupational Safety and Health Act**
  - A. Government Regulatory Agency
    - 1. Department of Labor: 29 Code of Federal Regulations Parts 1900-1910
      - i. Hazard Communication Standard
      - ii. Hazardous Waste Operations
      - iii. Occupational Exposure to bloodborne pathogens Standards
    - 2. Department of the Interior, Environmental Protection Agency: 40 Code of Federal Regulations Parts 200-399
      - i. Clean Air Act and Clean Water Act
      - ii. Toxic Substances Control Act
      - iii. Comprehensive Environmental Response, Compensation and Liability Act (CERCLA)
    - 3. Voluntary Agencies/Accrediting Agencies
      - i. The Joint Commission
      - ii. College of American Pathologist
      - iii. Centers for Disease Control and Prevention
      - iv. Clinical and Laboratory Standards Institutes

I. LABORATORY HAZARDS

A. Occupational Hazard

***Occupational Hazards are hazards and risks of illnesses or accidents in the workplace.*

1. Fire Hazards

- Enforcement of a non-smoking policy
- Placement of fire extinguishers every 75 feet, checked monthly and maintained annually.
- Placement of fire detection system and manual fire alarm near exit doors which is less than 200 ft away and should be tested every three months.
- Written fire prevention and response procedures and fire drills

***related to chemical and electrical hazards*
***emergency fire drills are important to avoid risks*
***REQUIRED inside facility:*
- ✓smoke detector system
- ✓automatic fire sprinkler
- ✓floor plan
  - -includes designated fire exits

Table 1.0: *Types of Fires and Fire Extinguishers*

FIRE TYPE	COMPOSITION OF FIRE	TYPE OF FIRE EXTINGUISHER	EXTINGUISHING MATERIAL
Class A	Wood, paper, or clothing	Class A	Water
Class B	Flammable organic chemicals	Class B	Dry chemicals, carbon dioxide, foam, or Halon
Class C	Electrical	Class C	Dry chemicals, carbon dioxide, or Halon
Class D	Combustible metals	None	Sand or dry powder
		Class ABC	Dry chemicals
Class K	Grease, oils, fats	Class K	Liquid designed to prevent splashing and cool the fire.

Table 2.0: ***IN CASE of Fire and Proper Use of Fire Extinguisher***

IN CASE OF FIRE	PROPER USE FIRE EXTINGUISHER
Rescue	**P**ull the pin
Activating fire alarm	**A**im the nozzle at the base of the fire
Containing the fire **closing window and doors*	**S**queeze the lever slowly
Extinguishing the fire	**S**weep from side to side

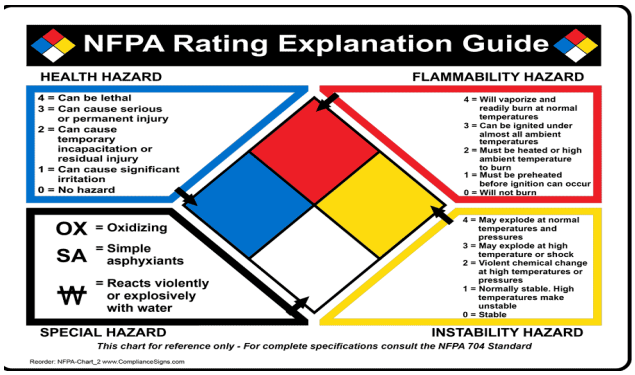
2. Chemical Hazards

- Labelling of all chemicals properly.
- Follow handling, storage requirements.
- Use adequate ventilation.
- Spill response procedures should be included in the safety procedures.
- MSDS should be available and reviewed by laboratory personnel.

***also refers to reagents, and acids used inside the laboratory.*
***storage of chemicals should be observed to avoid this hazards risk.*
- ✓always adequate ventilation

MSDS Diamond (Material Safety Data Sheet)

***also known as NFPA Rating Explanation Guide which contains the common identifier along with a rating number (from 0-4) inside a colored field to indicate hazard rating.*



BLUE -- HEALTH HAZARD
4 – Deadly
3 – Extreme Danger
2 – Hazardous
1 – Slightly Hazardous
0 – Normal Material

RED – FLAMMABILITY HAZARD
Flash Points
4 – Below 73°F
3 – Below 100°F
2 – Below 200°F
1 – Above 200°F
0 – Will not burn

WHITE – SPECIFIC HAZARD
Oxidizer – OX
Acid – ACID
Alkali – ALK
Corrosive – COR
Use no water – W
Radiation Hazard --

YELLOW – INSTABILITY HAZARD
4 – May detonate
3 – Shock and heat may detonate
2 – Violent chemical change
1 – Unstable if heated
0 -- Stable

3. Electrical Hazards

- Use of adapters, gang plugs and extension cords are prohibited
- Stepping on cords, rolling heavy equipment over cords should be prohibited
- Before repair or adjustment of electrical equipment, unplug first the equipment making sure that the hand is dry and no jewelry should be present

***most common hazard inside the laboratory*
***DIRECT – can cause shock or even*
INDIRECT – usually results to fire and explosion
✓machines or equipment should be near/close to the power source.
✗ using of electrical extensions

4. Sharp Hazards

- ❖ sharp objects such as syringe with needle, knives for histopathology analysis, and lancets.
- ❖ contaminated needle is dangerous (accidental pricks)

➢ Needle Puncture

- Containers should be puncture proof
- Improper disposal is the major cause of needle prick incident
- Replaced once the container is ¾ full

*** Fully- filled container is prohibited.*
*** 0.01%-0.1% chance of getting infection from needle stick injury*

B. Physical Hazard

- ❖ An agent, factor, or circumstance that can cause harm without contact.

It includes:

- Ergonomic Hazard
  - Can affect posture
- Vibration Hazard
  - From the analyzers
- Noise Hazard
  - From the analyzers or machines

C. Other Hazard

1. Biological Hazards

- ❖ Refers to the bacteria, fungi, parasites, and virus.
- ❖ Dealing with all microorganisms.
  ***Bacillus subtilis – most common type of contaminant*

2. Radiation Hazards

- ❖ Most common in radiology laboratory
  *** x-rays, MRI's*

II. BIOSAFETY LEVEL

LEVEL	RISK	TYPES OF AGENTS	EXAMPLES OF AGENTS	PRECAUTIONS
I	Minimal	Those not known to cause disease in healthy adults	*Bacillus subtilis Mycobacterium gordonae (tap water) soil microbes*	Standard microbiological practice. ***ASEPTIC TECHNIQUE* No special equipment. ✓ PPE's ✓ Good immune system
II	Moderate	Common human pathogens.	*E. coli, Salmonella, HIV, HBV, influenza*	Biolological Safety Cabinet (BSC) I or II. PPE's Autoclave Limited access Most microlabs fall in this category.
III	High	Those that may cause serious or lethal disease via inhalation. ***droplets **airborne* Effective treatment available.	*Bacillus anthracis, Francisella, Brucella, Mycobacterium tuberculosis, Rickettsia rickettsii, Coxiella burnetelli,* mold stages of systemic fungi	Same as above plus negative air flow, sealed windows. ✓proper ventilation
IV	Extreme	Those that pose high risk of life-threatening disease. May be transmitted by aerosols. NO VACCINE or THERAPY.	Ebola virus, Lassa virus, others that cause hemorrhagic fevers	Requires use of class III BSC; full-body air supplied positive pressure suit; independent unit with specialized ventilation & waste management to prevent release into environment ✓Biosafety Cabinet ✓ Waste Management Control ✓ proper ventilation

*** CoViD-19 lies between Level III and Level IV*

A. Safety Precautions

- Exposure to blood and body fluids is the most common risk associated in hematology laboratory.
- Bloodborne pathogens are pathogenic microorganisms present in blood causing infection or diseases.
  ***WHO, DOH*

1. OSHA Standards (Occupational Safety and Health Administration)

- OSHA provides standards to maintain safe work environment.
- The following practices are enforced inside the laboratory:
  1. Handwashing
  2. Food, drink and medications not allowed
  3. Applying cosmetics are prohibited
  4. Fomites or any surfaces must be kept away from mouth and all mucous membranes.
     ***FOMITES- surfaces that are likely to cause an infection.*
  5. Contaminated sharps must be disposed properly
  6. Personal Protective Equipment must be worn at all times following the proper donning
  7. Equipment should be check and maintained

***OSHA Philippines works hand-in-hand with DOLE*

Handwashing Procedure

- Wash your hands BEFORE: entering workplace, handling equipment, before filling up napkin dispensers, eating
- Wash your hands AFTER: going to toilet, meal, smoking, cleaning, handling wastes, removing gloves, touching parts of the body, every patient interaction, handling chemicals
- HOW TO WASH YOUR HANDS?

❖ Turn on tap, wet hands with warm water then apply liquid soap, lather and rub at least for 20 seconds.
❖ Clean each nail, between each finger, front and back of the hands up to the wrist then rinse off soap using water pointing downwards.
❖ Dry hands using disposable paper towel.
❖ Turn off the water tap using another disposable paper towel.

***Handwashing must be done for 1 minute or 10 seconds each step.*
***clothes should not touch any area of the sink*
***PROPER DONNING*
- *Handwashing*
  - ✓ Shoe Cover
  - ✓ Gown
  - ✓ Mask or Respirators
  - ✓ Goggles or Face Shield
  - ✓ Gloves

***PROPER DOFFING*
- ✓Gloves
- ✓ Goggles
- ✓ Gown
- ✓ Mask
- Handwashing

***Another DOFFING technique*
- ✓Gown and Gloves (for disposable gowns)
- Handwashing
- ✓ Goggles
- ✓ Mask
- Handwashing

III. LABORATORY WASTE MANAGEMENT

A. Segregation

❖ Separation of waste
1. Biodegradable
2. Non-biodegradable
3. Hazardous Waste
   - ✓Special waste
   - ✓Biological Waste
   - ✓Chemical Waste
   - ✓Radioactive Waste

*** AGAR disposal*
- *Disinfect*
- *Autoclave before disposal*
  *** autoclave is an essential tool inside the laboratory.*

B. Waste Disposal Standard

1. Blood Containing Waste

- Objects contaminated with blood should be autoclaved before disposal.
- Blood should be treated before disposal; treatment involves the use of aldehyde, chlorine compounds, phenolic compounds or thru autoclaving before pouring down the sink with running water.

Standard Waste Protocol (Philippines)

COLOR	TYPE OF WASTES
RED	Sharps, needles
YELLOW	Infectious wastes
YELLOW WITH BLACK BAND	Chemical Wastes
ORANGE	Radioactive Wastes
GREEN	Non-infectious wet wastes, biodegradable wastes
BLACK	Non-infectious dry wastes, non-biodegradable wastes

WASTE CONTAINER/BAGS ARE COLOR CODED
SOURCE: DEPARTMENT OF HEALTH

C. Disposal

❖ Flushing down the drain to the Sewer System
*** specific sewer system*
❖ Incineration
*** Level III or Level IV Biosafety level*
❖ Landfill burial
❖ Recycling

IV. Occupational Safety and Health Act

❖ Republic Act 11058 "An Act Strengthening Compliance with Occupational Safety and Health Standards and Providing Penalties for Violations Thereof"
*** Aims to make sure that all workers in any field are protected in all hazards.*

GOAL: Provide all employees (clinical laboratory personnel included) with a safe work environment.

A. Government Regulatory Agency

1. Department of Labor: 29 Code of Federal Regulations Parts 1900-1910
   - ❖ Hazard Communication Standard
   - ❖ Hazardous Waste Operations
   - ❖ Occupational Exposure to bloodborne pathogens Standards
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   - ❖ The Joint Commission
   - ❖ College of American Pathologist
   - ❖ Centers for Disease Control and Prevention (CDC)
   - ❖ Clinical and Laboratory Standards Institute.

Safety in the Laboratory is BASIC but needs COMMON SENSE

Blood Collection Technique

CLINICAL HEMATOLOGY 1

Instructor: Prof. Antonio C. Pascua, Jr., RMT, MSMT

Date: September 21, 2021

Outline

At the end of the session, the student must be able to learn:

- **I. Blood Collection Technique**
  - A. Arterial Puncture
    - 1. Puncture Sites
      - i. Radial Artery
      - ii. Brachial Artery
      - iii. Femoral Artery
    - 2. Complications
      - i. Arteriospasm
      - ii. Hematoma
      - iii. Nerve Damade
      - iv. Fainting
  - B. Skin Puncture
    - 1. Sites of Puncture
      - i. Finger
      - ii. Earlobe
      - iii. Lateral Portion of the Plantar Surface of the Heel or Toe
    - 2. Sites to avoid
    - 3. Indications of Skin Puncture
      - i.Newborn/Infant
      - ii. Geriatic
      - iii. Test that requires small amount of blood
    - 4. Advantages of Skin Puncture
    - 5. Order of Draw
    - 6. Complications
  - C. Venipuncture
    - 1. Things to Remember
    - 2. Method of Collection
      - i. Single Collection
      - ii. Multiple Colletion
      - iii. Winged Collection
    - 3. Sites of Puncture
    - 4. Sites to avoid
    - 5. Complications
    - 6. Venipuncture in Special Situations
    - 7. Additives in Collection Tubes
      - i. Clot Activators
      - ii. Anticoagulants
      - iii. Antiglycolytic Agent
      - iv. Separator Gel

I. BLOOD COLLECTION TECHNIQUE

***Blood is one of the most commonly submitted and processed specimens in the laboratory. Examination of blood in the different sections of the laboratory provides a picture of the condition of the patient. It is important that blood samples are collected and processed properly so that accurate, precise, and reliable results are obtained.*

***Regulations of the Occupational Safety and Health Administration (OSHA) that took effect on March 6, 1992, outlined in detail what must be done to protect health care workers from exposure to bloodborne pathogens, such as the pathogens that cause hepatitis C, hepatitis B, hepatitis D, syphilis, malaria, and human immunodeficiency virus (HIV) infection*

***Standard precautions must be followed at all times, with special attention to the use of gloves and hand washing.*

A. Arterial Puncture

Done to collect arterial blood which is used for blood gas analysis

Collected from an artery, primarily to determine arterial blood gases

Performed by: DOCTORS OR RESPIRATORY THERAPIST

Can be obtained either through a catheter placed in an artery or by using a needle and syringe to puncture an artery

- Performed in emergency cases: accident, sudden heart attack

- A catheter or using needle or syringe → must be PRE HEPARINIZED

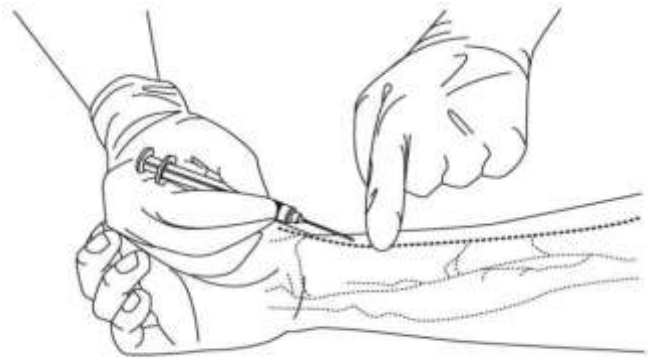
1. Puncture Site

- Radial artery
  - Thumb No need for tourniquet
  - 90 degree
  - Dangerous and critical
- Brachial artery - At arm. Harder to locate
- Femoral artery
-

2. Complications

- Arteriospasm - help them relax
- Involuntary contraction of the artery
- Hematoma
- Excessive bleeding
- Nerve damage - prevented by choosing an appropriate sampling site and avoiding redirection of the needle - select the appropriate veins for venipuncture
- Fainting - prevented by ensuring that the patient is supine with feet elevated before beginning the blood draw. “Supine position”

Figure 1.1



Locate artery and take a sample

B. Skin Puncture

A mixture of capillary, venous, and arterial blood with interstitial (tissue) fluid and intracellular fluid.

1. Sites of Puncture

- Finger - Non dominant, 3rd or 4th finger - 3-4 mm deep
- Earlobe - Abnormality in WBC (presence of callus)
- <1yo Lateral portion of the plantar surface of the heel or toe- ideal for newborn

2. Sites to Avoid

- inflamed and pallor areas
- cold and cyanotic areas
- congested and edematous areas
- scarred and heavily calloused areas

Figure 1.2

Table 7.1 Conditions influencing the choice of heel or finger-prick

Condition	Heel-prick	Finger-prick
Age	Birth to about 6 months	Over 6 months
Weight	From 3–10 kg, approximately	Greater than 10 kg
Placement of lancet	On the medial or lateral plantar surface	On the side of the ball of the finger perpendicular to the lines of the fingerprint
Recommended finger	Not applicable	Second and third finger (i.e. middle and ring finger); avoid the thumb and index finger because of calluses, and avoid the little finger because the tissue is thin

Image source: WHO Guidelines on Drawing Blood

3. Indications of Skin Puncture

- Newborn/infant - Less amount of blood
- Geriatric - To avoid collapsing
- Test requiring small amount of blood

4. Advantages of Skin Puncture

It is **easy to obtain** (it can be difficult to obtain blood from the veins, especially in infants). There are several collection sites on the body, and these sites can be rotated. Testing can be done at home and with little training.

5. Order of Draw

- Hematology- *platelets are not aggregated
- Clinical chemistry blood banking

6. Complications

•collapse of veins if the tibial artery is lacerated from puncturing the medial aspect of the heel
•osteomyelitis of the heel bone (calcaneus)
•nerve damage if the fingers of neonates are punctured
•haematoma and loss of access to the venous branch used
•scarring
•localized or generalized necrosis

•skin breakdown from repeated use of adhesive strips

C. Venipuncture

•manner of inserting a needle attached to a syringe to a palpable vein to collect blood for laboratory testing
•Specimen collected: venous blood
•Most widely used blood sample in all laboratory tests

collecting venous blood

1. Things to Remember

• Proper identification of patient
• Tourniquet application- only 1 min, 3-4 inches away used to provide a barrier against venous blood flow to help locate a vein.
***A tourniquet can be a disposable elastic strap, a heavier Velcro strap, or a blood pressure cuff. Latexfree tourniquets are available for individuals with a latex allergy.*
• Disinfection
• Angle of needle insertion- 30-40 degree
• Bevel UP
• Needle length
• Position of the patient
• Label
• Disposal

2. Method of Collection

- Single Collection - Syringe collection - consists of a barrel, graduated in milliliters, and a plunger.
- Multiple Collection - ETS. reduces the risk exposure of blood.
***OSHA recommends the use of plastic tubes whenever possible. Most glass tubes are coated with silicone to help Decrease the possibility of hemolysis and to prevent blood from adhering to the sides of the tube.*
• "Winged" Collection - Butterfly set

Figure 1.3

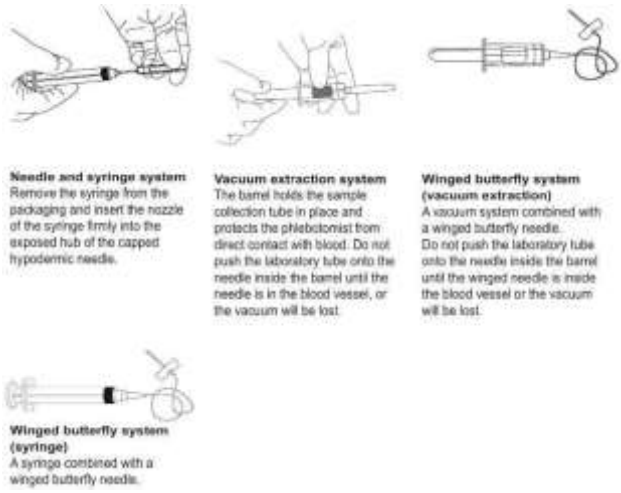


Image source: WHO Guidelines on Drawing Blood

Figure 1.4

Table 3.1 Recommended needle gauge, length and device for routine injection and phlebotomy procedures for different age groups

Needle gauge	Patient population: Adult	Patient population: Paediatric, elderly, small veins	Patient population: Neonatal	Procedure
16–18	√			√ Blood donation
19–20	NA	NA	NA	
21	√ (1–1.5 inch or 2.54 cm)	NA	NA	
22	√ (1 inch or 2.54 cm)	√ (1 inch or 2.54 cm)	NA	
23	√ (1–1.5 inch or 2.54 cm)	√ (Winged set [butterfly]; 0.5 inch or 0.75 cm)	√ (Winged set [butterfly]; 0.5 inch or 0.75 cm)	

NA, not applicable

Image source: WHO Guidelines on Drawing Blood

3. Sites of Puncture

***Needles for drawing blood range from 19 to 23 gauge.*
***The most common needle size for adult venipuncture is 21 gauge with a length of 1 inch*

- Median - Most stable - connects the basilic and cephalic veins in the antecubital fossa
- Cephalic - Lateral area "away" - Blood spurts. - Quiet unstable - the cephalic vein, located on the outside (lateral) aspect of the antecubital fossa on the thumb side of the hand;
- Basilic - Easily rolls - More chances of hematoma - w/ nerve endings - the basilic vein, located on the inside (medial) aspect of the antecubital fossa
- Occluded veins - Multiple puncture part - Impared circulation
- Fistula - Abnormal connection - Result from injury/surgery. - Connection of organs/vessel - Dialysis patient
- IV fluid site - If no choice at all. - Stop for 5-10 mins - First 5 ml, disregard

Older children (18months to 3 yo)
• Femoral vein
• Long saphenous vein
• Popliteal vein
•Ankle vein *Antecubital fossa

Figure 1.5





3 yo to adult life
• Wrist vein
• Dorsal vein of hand
• Dorsal vein of ankle *Antecubital fossa

Figure 1.6

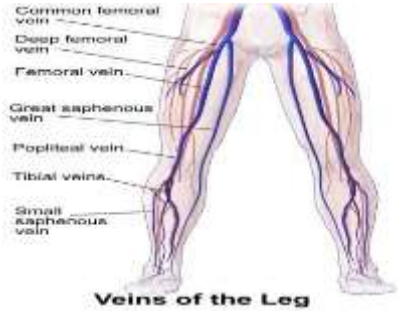
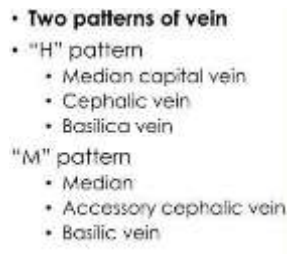


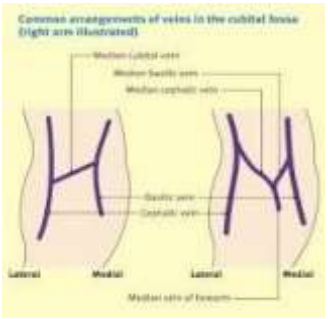
Figure 1.7



Antecubital fossa
• Two patterns of veins
1. H pattern - Median capital vein - Cephalic vein - Basilic vein
2. M pattern - Median - Accessory cephalic - Basilic vein

Figure 1.8





*** The cephalic and basilic veins should only be used if the median cubital or median veins are not prominent after checking both arms. The basilic vein is the last choice due to the increased risk of injury to the median nerve and/or accidental puncture of the brachial artery, both located in close proximity to the basilic vein.*

4. Sites to Avoid

• Sites with hematoma
• Occluded veins
• Edematous area
• Sites with burns, scar, tattoo
• Sites with Fistula
• IV fluid sites

5. Complications

- Ecchymosis (Bruise)
  -most common complication. Caused by leakage of a small amount of blood in the tissue around the puncture site
  -can prevent applying direct pressure to the venipuncture site with a gauze pad
  -Bending the patient's arm at the elbow to hold the gauze pad in place is not effective in stopping the bleeding and may lead to bruising.
- Hematoma
  -a results when leakage of a large amount of blood around the puncture site causes the area to rapidly swell.
  -remove the needle immediately and apply pressure at least 2 minutes.
  -may result in bruising of the patient's skin around the puncture site.
  -can also cause pain and possible nerve compression and permanent damage to the patient's arm.
  -most commonly occur when the needle goes through the vein or when the bevel of the needle is only partially in the vein and when the phlebotomist fails to remove the tourniquet before removing the needle or does not apply enough pressure to the site after venipuncture.
  -can also form after inadvertent puncture of an artery.
- Pain
- Syncope and fainting
- Iatrogenic anemia
- Infections
- Edema
- Allergies
  -use hypoallergenic tape or apply pressure manually until the bleeding has stopped completely
- Petechiae
  -small red spots indicating that small amounts of blood have escaped into the skin.
  -indicate a possible hemostasis abnormality and should alert the phlebotomist to be aware of possible prolonged bleeding.
- Hemoconcentration
  -increased concentration of cells, larger molecules, and analytes in the blood as a result of a shift in water balance
  -caused by leaving the tourniquet on the patient's arm for too long. The tourniquet should not remain on the arm for longer than 1 minute. If it is left on for a longer time because of difficulty in finding a vein, it should be removed for 2 minutes and reapplied before the venipuncture is performed
- Hemolysis
  - rupture of red blood cells with the consequent escape of hemoglobin—a process termed hemolysis—can cause the plasma or serum to appear pink or red

Table 1.1

Adverse event	Cause	Management
Hematoma	Poor collection techniques	Apply pressure and firm badge
Fainting due to: a hypothalamic response resulting in bradycardia, vomiting, sweating	Anxiety Lowered blood volume and other associated causes	Recline chair Discontinue collection Loosen clothes Give fluid
Syncope	Physical stress Inadequate fluid intake	Fluid administration

6. Venipuncure in Special Situations

- Edema Swelling caused by an abnormal accumulation of fluid in the intercellular spaces of the tissues is termed edema. The most common cause is infiltration of the tissues by the solution running through an incorrectly positioned intravenous catheter. Edematous sites should be avoided for venipuncture because the veins are hard to find and the specimens may become contaminated with tissue fluid.
- Obesity In obese patients, veins may be neither readily visible nor easy to palpate. Sometimes the use of a blood pressure cuff can aid in locating a vein. The cuff should not be inflated any higher than 40 mm Hg and should not be left on the arm for longer than 1 minute.9 The phlebotomist should not probe blindly in the patient's arm because nerve damage may result.
- Burned, Damaged, Scarred, and Occluded Veins Burned, damaged, scarred, and occluded veins should be avoided because they do not allow the blood to flow freely and may make it difficult to obtain an acceptable specimen.
- Mastectomy Patients The CLSI requires physician consultation before blood is drawn from the same side as a prior mastectomy (removal of the breast), even in the case of bilateral mastectomies.9 The pressure on the arm that is on the same side as the mastectomy from a tourniquet or blood pressure cuff can lead to pain or lymphostasis from accumulating lymph fluid. The other arm on the side without a mastectomy should be used.

7. Additives in Collection Tubes

- **Clot activators**. Blood specimens for serum testing must first be allowed to clot for 30 to 60 minutes prior to centrifugation and removal of the serum.10 A clot activator accelerates the clotting process and decreases the specimen preparation time. Examples of clot activators include glass or silica particles (activates factor XII in the coagulation pathway) and thrombin
- **Anticoagulants**. Prevents blood from clotting.
    - **Ethylenediaminetetraacetic acid (EDTA), citrate, and oxalate** remove calcium needed for clotting by forming insoluble calcium salts.
    - **Heparin** prevents clotting by binding to antithrombin in the plasma and inhibiting thrombin and activated coagulation factor X.

** Tubes with anticoagulant must be gently inverted immediately

- **Antiglycolytic Agent.** Inhibits the metabolism of glucose by blood cells. Such inhibition may be necessary if testing for the glucose level is delayed. The most commonly used antiglycolytic agent is sodium fluoride.
    - Tubes containing sodium fluoride alone yield serum.
    - Tubes containing sodium fluoride and an anticoagulant (such as EDTA or oxalate) yield plasma
- **Separator Gel**. Is an inert material that undergoes a temporary change in viscosity during the centrifugation process; this enables it to serve as a separation barrier between the liquid (serum or plasma) and cells. Because this gel may interfere with some testing, serum or plasma from these tubes cannot be used with certain instruments or for blood bank procedures.

Red Blood Cell Count

CLINICAL HEMATOLOGY 1

Instructor: Prof. Antonio C. Pascua, Jr., RMT, MSMT

Date: September 29, 2021

Outline

At the end of the session, the student must be able to learn:

- **I. RED BLOOD CELL COUNT**
- **II. MATERIALS NEEDED (MANUAL)**
  - A. Hemacytometer
  - B. Thoma Pipette
  - C. Suction Device
  - D. Thick Coverslip
  - E. Cell Counter
  - F. RBC Diluting Fluid
- **III. PROCEDURE**
  - A. Additional Procedure
- **IV. POST LABORATORY**
- **V. SOURCES OF ERRORS AND COMMENTS**
- **VI. NORMAL VALUES**

I. RED BLOOD CELL COUNT

- *The number of WBCs in 1 liter or 1 microliter of blood*
- *Manual RBC counts are rarely performed because of the inaccuracy of the count and questionable necessity. Use of other, more accurate manual RBC procedures, such as the micro hematocrit and hemoglobin concentration, is desirable when automation is not available.*

• Highest in the morning and lowest in the evening.
• **Increased:** myeloproliferative disorder such as polycythemia
• **Decreased:** anemic cases, bleeding
***counting of red blood cells will give us the reflection of how our body is capable of delivering oxygen.

- ***ELEVATION OF RBC COUNT** = excessive production of bone marrow Manual cell counts are performed using a hemocytometer for counting chamber, and manual dilutions made with calibrated, automated pipettes and diluents (commercially available or laboratory prepared). The principle for the performance of cell counts is essentially the same for white blood cells, red blood cells, and platelets; only the dilution, diluting fluid, and area counted vary.
- Will not confirm any forms/ types of diseases. Only a **SCREENING TEST**
- Identifying how capable the body is to transport oxygen to different parts of our body.

II. MATERIALS NEEDED (MANUAL)

***COMPLICATED AND SUBJECTIVE*

- Hemacytometer
- Thoma Pipette
- Suction device
- Thick coverslip
- Cell Counter
- Diluting fluids

1. HEMACYTOMETER

More convenient, accurate, organize

•"Heart of manual cell count"
•Different Types

- ***Improved Neubauer***
- Neubauer
- Fuchs –Rosenthal
- Speirs–Levy -Tuerk's
- Bass –Jones

** *The most commonly used counting chamber is the Levy chamber with improved Neubauer ruling.*
**Thoma pipette is also essential in manual cell counting procedures to accurately dilute blood specimens with fluids needed for specific cell counting procedures. A red cell pipet and a white cell pipet are the thoma pipets used in cell counting. A red cell pipet can contain 101units of volume whereas white cell pipet can contain 11units.*

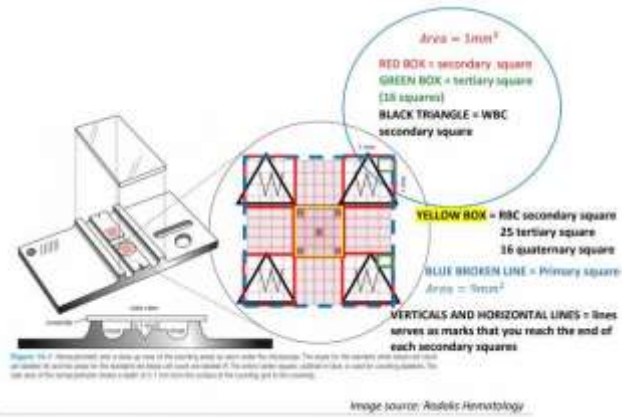


Image source: Rodaks Hematology

Primary square: 2
Each primary square has 9 SECONDARY SQUARE
The four corners of the secondary square is for WBC
WBC secondary square has 16 TERTIARY SQUARE.
For RBC in the center has 25 TERTIARY SQUARE
Each 25 TERTIARY SQUARE has 16 QUATERNARY SQUARE.
**each secondary square has 1mm x 1mm ➔ Total of $9mm^2$

2. RBC DILUTING FLUID

• NSS
• 3.8% Na citrate
• Dacies
• Hayem's
• Toisson's
• Bethell's
• Gower's
****ISOTONIC solution (RBC)*
****HYPOTONIC (WBC)*

III. PROCEDURE

1. Draw blood up to 0.5 mark using the RBC Pipette.
   Note: if the blood was drawn too far above the 0.5 mark, the procedure should be repeated using a new pipette, excess blood causes an inaccurate result.
2. Wipe the outside walls of the pipette with clean gauze.
   Note: do not allow capillary attraction to draw fluid from the tip onto the gauze. Gauze tends to absorb the liquid portion of the blood, causing an inaccurate result.
3. Dip the pipette into diluting fluid, and then draw the diluting fluid into the pipette, slowly, until the mixture reaches the 101 mark. Gently rotate the pipette to ensure a proper amount of mixing. The dilution is 1:200
4. Remove the tubing from the pipette, and then mix it on a horizontal axis for 5 minutes.
5. Discard the first 3-4 drops of the diluted sample.
6. Charge both sides of the hemocytometer counting chamber with a drop of the diluted sample and allow to stand for few minutes.
7. While keeping the hemacytometer in a horizontal position, place it on the microscope stage.

8. Using HPO, count the red cells in the 5R's squares of the central secondary square.
9. Calculate the number of RBC per luter of each of the hemocytometer. Average the results and report this number.

*** additional procedure:

STEP 1: ***directly aspirate the blood from EDTA tube

STEP 3: The mixture will confine at the bulb
**** no bubbles
**** no spaces

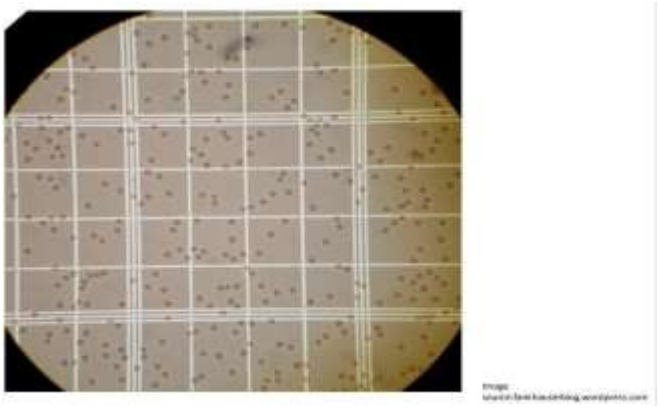
STEP 4: *** 5 minutes mixing

STEP 5: discard the first 3-5 drops

STEP 6: *** put the slide to petri dish with moist tissue paper or gauze to keep the moisture (5 – 10 minutes incubating)

STEP 7: ***microscope
*** PARFOCAL FOCUSING
*** Scanner = primary square
LPO = secondary square
HPO = tertiary square

STEP 8: *** cells touching LEFT and TOP triple lines are included in count
Cells touching RIGHT and



BOTTOM triple lines are NOT included in count

****aspirate blood directly from EDTA tube

Rules

- count all cells inside the square
- count all the cells in the middle of L line " L RULE"

***the difference between each primary square must only be 5-10%

IV. POST LABORATORY

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor}}{\text{area (mm}^2\text{)} \times \text{depth (0.1)}}$$

Or

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor} \times 10^n}{\text{area (mm}^3\text{)}}$$

***** CELLS COUNTED = get the AVERAGE RBC count of 2 counting area (top and bottom)**

DILLUTION FACTOR = solute ÷ total volume

DEPTH = constant value 0.1 mm

Area mm^2 = RBC area you count

**** RBC tertiary square 1^{st}, 5^{th}, 13^{th}, 21^{st}, 25^{th}**

(i.e every tertiary square= 1 (tertiary area) ÷ 25 (squares)) = 0.04

0.04 x 5 (number of RBC area you count) = 0.02 (area factor

Unit for Final RBC Count ($x10^{12}$/L)

*****rule of 3 is only applicable for normocyctic and normochromic**

V. SOURCES OF ERRORS AND COMMENTS

- Dust and fingerprints may cause difficulty in distinguishing the cells
- Diluting fluid should be free of contaminants
- If the count is low, a greater area may be counted
- Chamber must be charged properly to ensure accurate count
- Allow cells to settle for 10mins before counting
- Use of other, more accurate manual RBC procedures, such as the microhematocrit and hemoglobin concentration, is desirable when automation is not available

VI. NORMAL VALUES

- Female: 3.6 – 5.6 x 1012/L
- Male: 4.2 – 6.0 x 1012/L
- At Birth: 5.0 – 6.5 x 1012/L

Outline

At the end of the session, the student must be able to learn:

I. **Hemoglobin**
II. **Methods**
   i. Acid Hematin
   ii. Cyanmethemoglobin
   iii. Other Methods

I. Hemoglobin

Hemoglobin is the most significant component of RBC.

- Globin
- Protoporphyrin IX
- Iron

- Higher in the morning (due to physical activities)
- Lower in the evening (resting time)
- Carries oxygen from the lungs to the tissues and takes carbon dioxide
- Screen for anemia and may detect RBC breakdown/ hemolytic anemia
- Higher in high altitude
- Increase in strenuous muscular activity

Normal Values:

- At birth: 15-20 g/dL
- Women: 12-16 g/dL
- Men: 13-18 g/dL

High	Low
Sickle cell anemia	Anemia
Thalassemia	Blood loss
Transfusion reaction	Iron, folate and Vitamin B6, B12 deficiencies
Hemolysis	
Dehydration	
Polycythemia vera	
High altitude	

II. Methods

1. Acid Hematin

Principle: Colorimetric

Comparator block: Subjective

***Compare the "color" of solution to standard

Materials and Instruments

A. Specimen
   Capillary or unclotted venous blood
B. Hemoglobinometer or Ohmmeter set consisting of:
   1. Comparator block or color standard
   2. Sahli pipette – marked 20 cu.mm. or 0.02 cc.
   3. Sahli graduated tube – calibrated grams on one side of the scale and percentage on the opposite scale
   4. Stirrer – used to mix instead of shaking to minimize bubble on the surface of the solution 5. Dropper
   6. Distilled water
   7. 0.1 N HCl solution

****Sahli pipette**: Straight micropipette with a single mark = 0.02 mL blood*

***Sahli graduated tube**: with markings with an increment of 2 every line that is equivalent to 0.2 (for blood and HCl)*

Procedure

1. Introduced 0.1 N HCl solution up to 2 mark of the Sahli graduated tube
2. Using the Sahli pipette, draw blood up to 0.02 cc mark. Observe the same technique as in the use of WBC or RBC pipette.
3. Dispense the measured volume of the blood into the graduated tube. Gently draw up and down to rinse the pipette and also to mix the resulting colloidal suspension.
4. Thoroughly mix with the use of the stirrer and allow to stand for 5 minutes.
5. Add distilled water drop by drop, stirring after each addition and compare with the comparator block. 6. When the resulting solution compares with the color standard in the comparator block, get the reading either from the grams or percentage scale.
7. Write your result.

****Equivalent Hgb value concentration:** check the line in graduated tube*

Brown color* = *red cells have been lysed.

2. Cyanmethemoglobin

Principle:

potassium ferricyanide — potassium cyanide

$Hb\ (Fe^{+2}) \rightarrow$ methemoglobin $(Fe^{+3}) \rightarrow$ cyanmethemoglobin

- Absorbance of cyanmethemoglobin at 540 nm (set wavelength) is directly proportional to the Hb concentration
- Uses a standard curve (spectrophotometer)
  ****indirect colorimetric*
  ****manually or automated is still acceptable*

Materials and Instruments

- Anticoagulated blood (venous blood)
- Test tubes
- Pipette
- Spectrophotometer
- Drabkin's Reagent
- Parafilm

Procedure

1. Place 5 cc of Drabkin's reagent into a test tube.
2. Using the Sahli pipette, draw blood to 0.02 cc mark. Make sure to wipe the outer wall of the pipette, and then dispense the blood to the test tube.
3. Gently draw up and down to rinse the pipette and also to mix the resulting colloidal suspension. NOTE: The pipette should be rinsed thoroughly with the reagent to ensure that no blood remains. 4. Cover and mix well by inversion. Let it stand for 5 minutes.
5. Transfer the mixture to a cuvette.
6. Set the spectrophotometer to 100% transmittance at the wavelength of 540 nm, using a cyanmethemoglobin reagent as a blank.
7. Continue reading the patient's sample, and record the percentage transmittance. 8. The hemoglobin concentration is obtained from a calibration curve prepared with the use of standards. (Stable standard cyanmethemoglobin solution of concentration representing 1:250 dilution of whole blood containing 5, 10, and 15 grams of hemoglobin/100 mL are available commercially for calibration purposes and for periodic check-up on the accuracy of the colorimeter.

Reagent: Drabkin's Reagent

- Potassium ferricyanate
  Hgb === methemoglobin === cyanmethemoglobin
  ***Non-ionic detergent is for RBC lysing*
  *** Whatever absorbance you get is not yet the final hemoglobin concentration; needs to be compared with standard curve*
  Ab's = curve
  All forms of derivatives can be measured by cyanmethemoglobin except SULFHEMOGLOBIN

  ****Turbidity increases; absorbance of light also increases***

Sources of Error

1. Cyanmethemoglobin reagent is sensitive to light. It should be stored in a brown bottle or in a dark place.
2. A high WBC count (greater than 20×10^9/L) or a high platelet count (greater than 700×10^9/L) can cause turbidity and a falsely high result. In this case, the reagent-sample solution can be centrifuged and the supernatant measured.
3. Lipemia also can cause turbidity and a falsely high result. It can be corrected by adding 0.01 mL of the patient's plasma to 5 mL of the cyanmethemoglobin reagent and using this solution as the reagent blank.
4. Cells containing Hb S and Hb C may be resistant to hemolysis, causing turbidity; this can be corrected by making a 1:2 dilution with distilled water (1 part diluted sample plus 1 part water) and multiplying the results from the standard curve by 2.
5. Abnormal globulins, such as those found in patients with plasma cell myeloma or Waldenström macroglobulinemia, may precipitate in the reagent. If this occurs, add 0.1 g of potassium carbonate to the cyanmethemoglobin reagent. Commercially available cyanmethemoglobin reagent has been modified to contain KH_2PO_4 salt, so this problem is not likely to occur.
6. Carboxyhemoglobin takes 1 hour to convert to cyanmethemoglobin and theoretically could cause erroneous results in samples from heavy smokers. The degree of error is probably not clinically significant, however.
7. Because the hemoglobin reagent contains cyanide, it is highly toxic and must be used cautiously. Consult the safety data sheet (Chapter 2) supplied by the manufacturer. Acidification of cyanide in the reagent releases highly toxic hydrogen cyanide gas. A licensed waste disposal service should be contracted to discard the reagent; reagent-sample solutions should not be discarded into sinks.
8. Commercial absorbance standards kits are available to calibrate spectrophotometers.
9. Handheld systems are commercially available to measure the hemoglobin concentration. An example is the HemoCue[4,5] (HemoCue, Inc., Brea, CA) (Figure 14-19) in which hemoglobin is converted to azidemethemoglobin and is read photometrically at two wavelengths (570 nm and 880 nm).

3. Other Methods

Gasometric (Van Slyke oxygen capacity)

- 1 gm hb = 1.34 mL O2

Specific Gravity method (Copper sulfate)

- sp.gr = 1.053
- approximation of hemoglobin concentration
  15 seconds until the RBC blood drop settles at the bottom indicates normal hemoglobin
  ****the heavier the blood, hemoglobin is normal*

Chemical (Kennedy's, Wong's)

- 1gm Hb = 3.47 mg Iron

References:
- Rodak's Hematology 6th Edition
- Turgeon Clinical Hematology Theory and Procedures 5th Edition
- Laboratory Sidenotes of Prof. Antonio C. Pascua Jr., RMT, MSMT - OLFU Valenzuela CMLS

Hematocrit

CLINICAL HEMATOLOGY 1

Instructor: Prof. Antonio C. Pascua, Jr., RMT, MSMT

Date: November 2, 2021

Outline

At the end of the session, the student must be able to learn:

- **I. Hematocrit**
  - A. Principle
- **II. Method for Hematocrit Determination**
  - A. Macrohematocrit
    - Disadvantages
    - Materials
    - Procedures
  - B. Microhematocrit
    - Centrifugation
    - Materials
    - Procedures
- **III. Sources of Error**
- **IV. Trapped Plasma**
- **V. Normal Values**

I. Hematocrit

Ratio of the volume of packed red blood cells to the volume of whole blood.
Packed cell volume means RBC's only
Reported as percentage of L/L

Example 0.45 – 45%
0.47 – 47%
0.40 – 40%
SI UNIT for Hct is L/L

- Also an important parameter to differentiate anemia.
- The least choice parameter is RBC count.
- Priority would always be HCT and HGB
- Amount of space occupied by RBC's in a given volume of blood.

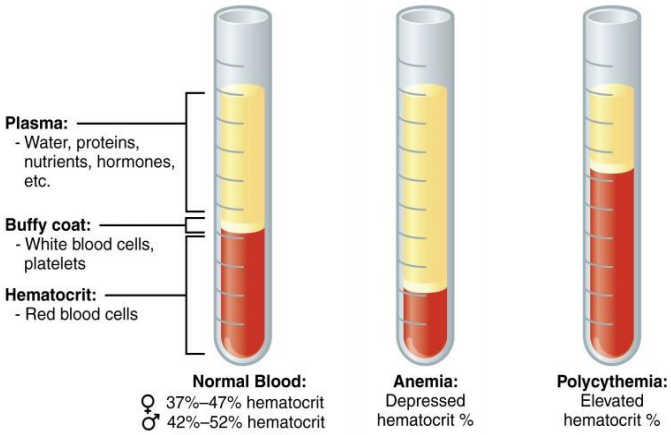
A. Principle

- Whole blood is centrifuged top determine packing of Red Blood Cells.
- When blood is centrifuged, blood components are separated depending on their densities.
- Two main divisions of blood are the solid and liquid part. The solid part I composed of the formed elements, RBC, WBC, and Plt. The liquid portion is composed of Plasma.
- At the top is plasma with water and other chemical constituents of the blood. The solid portion of blood is heavier/denser thus settles at the bottom of the tube. Among the 3 formed elements, the densest is the RBC since it has Hgb which contains oxygen.
- Buffy coat is composed of all other elements of the blood. At the top of the buffy coat is the platelets, then the monocytes, lymphocytes, the granulocytes which is heavier among the first two because of the granules and at the bottom of the buffy coat are the reticulocytes or if there are any immature blood cells.
- On hematocrit, we ONLY measure the RED BLOOD CELLS. We DO NOT INCLUDE the buffy coat, as it name implies PACKED RED CELLS.
- Hct is only a computated value for automated analyzer. In manual determination it depends on the RBC Count and Mean Cell Volume, and can be measure through macro and micro method.

II. Method for Hematocrit Determination

1. Macrohematocrit Determination

- You will be using larger amount of blood. Venipuncture then transfer to EDTA Tube.
- Whole Blood is transferred to a Wintrobe tube and centrifuged for 30 minutes at 2000-3000 rpm.
- **Wintrobe tube**- a special container for the measurement of ESR (0-10 down) and Hct (0-10 up). This is on a single tube thus, it has 2 markings. You just have to fill the tube with blood up to the 10th mark.



i. Disadvantages

The problem with this is, it is time consuming. It must be centrifuged for 30 minutes. Also large amount of blood must be used. As you centrifuge the blood, there will be trapped plasma in the container so this amount of plasma could still remain on the RBC portion of the blood after you centrifuged it.
If there is a left over plasma in between, Hct will be falsely elevated. So this is a common problem in the macro method.

a. **Wintrobe and Landsberg:** double oxalate (most common is the use of Wintrobe tube)
b. **Van Allen:** sodium oxalate
c. **Haden :** sodium oxalate
d. **Sandford-Magath:** sodium oxalate
e. **Bray:** heparin

The length of the Wintrobe tube is 115 mm and the diameter of the bore is 3mm.

ii. Materials

Wintrobe tube with rubber caps
Long stem pipette
Centrifuge
Anticoagulated blood (at least 3 mL of whole blood
Cotton

iii. Procedures

1. Mix the blood
2. With the use of a long stem pipette, full the Wintrobe tube to the10 mark

Note: No air bubbles should be present on the surface of the blood, it may be removed by touching it with tissue paper or with a pipette. If the bubbles are present in the middle of the tube, aspirate the blood and proceed to step 2 again.

3. Centrifuge the blood for 30mins.
4. Read the height of the packed red blood cells on the scale at the right side of the tube, which is graduated from 0-10 cm from the bottom to top, read upward.

If the packed RBC is in the 3.5 mark, multiplyby 10 then your Hct will be 35% or 0.35.
There are times that there a presence of slant RBC so make sure that you only include those parallel to the line.

2. Microhematocrit Determination

- Less blood, skin puncture only.
- Specimen is EDTA Blood (directly obtained)
- Capillary blood
- Sample is transferred to a capillary tube and centrifuged to a microhematocrit centrifuge.

Note: If you are going to use blood from EDTA tube, use a capillary tube with a blue mark (non-heparinized/no anticoagulant)

If you are going to use blood from skin puncture, use a capillary tube with red band (heparinized).

i.Centrifugation

- Better method for Hct.
- Heavier centrifugal force means more packed RBC.
- Heavier centrifugal force will avoid unwanted trapped plasma.
- Ten thousand to Fifteen thousand gravitational force, centrifuged for only 5-10 mins.
- 75 mm length with 1 mm bore diameter
- A lipemic patient will have lipid components on the top, but in most cases, only the plasma is present.
- A specialized centrifuge for microhematocrit could be used, it is called READACRIT – a centrifuge with markings/lines.

Microhematocrit Reading

- **Horizontal line-** align the end portion of packed cell. Do not include the clay or wax. Align at 0.
- **Diagonal line-** Align the last portion of the plasma here.
- **Adjustable diagonal line-** adjust this line to the packed cell, exluding the buffy coat.

Note: Make sure that before you put the capillary tube in the centrifuge, seal one end with wax, but clay will do.

Example:
The reading is 32, then you will report it as 32%. The accepted value for the reading could be less than or more than one. So 31% and 33% is accepted.

ii. Materials

Heparinized capillary tube
Microhematocrit reader
Microhematocrit centrifuge
Lancet
Cotton
Alcohol

iii. Procedures

1. After necessary preparation, perform a capillary puncture and produce a rounded drop of blood.
2. In a horizontal position, put one end of capillary tube on the drop of blood and fill the tube for about ⅔ full. Note: The tube will be filled with a capillary action. If using tubes with colored ring at one end, fill from the opposite end.
3. Seal one end of the capillary tube with clay by placing the dry end of the tube into the clay in a vertical position.
4. After sealing with clay, seal it again with wax.
5. Assemble the tube in the microhematocrit centrifuge in such a way that the unsealed end is near the center of the centrifuge.
6. Spin at 10,000 to 15,000 rpm for 5 mins
7. Using the microhematocrit reading device, determine the Hct. Note: Exclude the buffy coat in determining the Hct.
8. The reading on the window of the Hematocrit reader corresponds to the Hct value.

DO NOT FORGET TO WIPE OFF THE FIRST DROP OF BLOOD, AS TISSUE JUICES MAY INTERFERE.

III. Sources of Error

Increased	Decreased
• **Insufficient centrifugation time**- RBC will not be packed • **Inclusions of buffy coat** • **Disorders such as sickle cell anemia, marcocytic anemia, hypochromic anemia** • **Dehydration-** portion of plasma is water, if the patient is dehydrated then it will yield lesser plasma.	• **Improper sealing of the capillary tube-** there could be leakage. • **Increased concentration of anticoagulant-** EDTA can cause crenation or destruction of RBC. • **Prolonged centrifugation-** RBC may be destroyed • **Acute blood loss**

Additional note: During blood collection process, prolonged application of tourniquet may cause falsely increased Hct due to Hemoconcentration. For any reason that you may had difficulty in blood collection, interstitial fluid may interfere into the specimen and may cause falsely decreased Hct.

In cases of anemia, there is nothing we could do about it happens naturally.

IV.Trapped Plasma

Amount of plasma that still remains in RBC portion after the microhematocrit has been spun. This could increase the Hct by 1-3%.

V. Normal Values

At birth: 24-60%
Female: 36-48%
Male 40-55%

At the end of the session, the student must be able to learn:

I. **Erythrocyte Sedimentation Rate**
   a. Principle
   b. Stage of ESR
   c. Methods of ESR
   d. Procedure (Westergren)
   e. Procedure (Wintrobe)
   f. Sources of Error
   g. Automated ESR

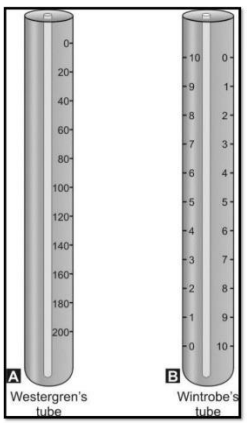
II. **Osmotic Fragility Test**
   a. Principle
   b. Materials
   c. Procedure (Sanford Method)
   d. Reference Value
   e. Data and

I. ERYTHROCYTE SEDIMENTATION RATE

- The **non-specific measurement used to detect and monitor and inflammatory response** to tissue injury.
- Used for screening on any possible inflammation. Termed also as **Sedimentation Rate-**measures the rate of setting of the RBC in diluted human plasma or whole blood.
- Ordered with another test to detect inflammatory conditions.
- May include RA, with certain infections, malignancy.
- It is not recommended for asymptomatic individuals because of its low specificity and sensitivity.
- Distance in millimeters at which the RBS's fall in 1 hour.

A. Principles

- When anticoagulated blood is allowed to stand at room temperature for a period of time, the RBC settle towards the bottom of the tube.
- If the tube is left undisturbed, RBC's will settle down. This will be reported in mm.
- Graduation found in Wintrobe and Westregren is in mm.
- The phenomenon depends on an interrelationship of variables, such as the plasma protein composition, the concentration of erythrocytes and the shape of RBC.
- You can have the plasma component of the blood.
- ESR will be affected by the RBC's. Since it has negative charge, they'll repel one another and this phenomenon is called **zeta potential**. This is partial or totally counteracted if there is increase in quantities of positively charged proteins on plasma, RBC will settle down rapidly.
- Faster Sedimentation Rate means higher ESR.



	WESTERGREN TUBE	WINTROBE TUBE
LENGTH	300 mm +/- 0.5 mm	115 mm or 11.5 cm
INTERNAL BORE	2.65 mm +/- 0.5 mm	3 mm
EXTERNAL BORE	5.5 mm +/- 0.5 mm	
GRADUATION/ MARKINGS	200	100

B. Stage of ESR

1. Initial rouleaux formation:10 mins

*RBC form a stock of coin. The more protein in plasma, the more promotes Rouleaux Formation.

2. Rapid Settling of RBC's: 40 mins
3. Final Sedimentation of RBC's: 10 mins

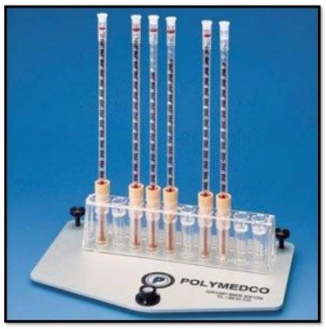
**total of 60 mins. ESR should be read on 60 mins time, less than 60 could cause falsely decreased result, more than 60 could cause falsely elevated result.

C. Methods of ESR

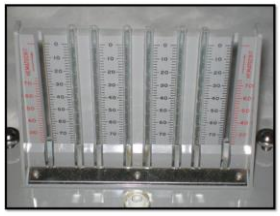
- **Westergren Method-** ratio is 4 volume of blood to 1 volume of sodium citrate on black top tube(9:1 from blue top tube). Most commonly used method for ESR. **Recommended by the international Council for the Standardization on Hematology and the CLSI.**
- **Modified Westergren**- EDTA is the anticoagulant used, it must be dilutred to the ratio of 4 volume of blood to 1 volume of 0.9% sodium citrate.
- **Wintrobe**- oxalate-anticouagulated whole blood



WINTROBE TUBE



WESTERGREN TUBE



WINTROBE ESR RACK



WESTERGREN ESR RACK

D. Procedure (Westergren)

Procedure

1. Mix the blood citrate or blood-EDTA-saline mixture thoroughly.
2. Aspirate a bubble-free specimen into a clean and dry Westergren pipette. Fill to the zero mark. Do not pipette by mouth.
3. Place the pipette into the vertical rack at 20°C to 25°C in an area free from vibrations, drafts, and direct sunlight.
4. After 60 minutes, read the distance in millimeters from the bottom of the plasma meniscus to the top of the sedimented erythrocytes.
5. Record the value as millimeters in 1 hour.

Clinical conditions	Acute heavy metal poisoning Acute bacterial infections Collagen vascular diseases Diabetes mellitus End-stage renal failure Gout Malignancy Menstruation Multiple myeloma Myocardial infarction Pregnancy Rheumatic fever Rheumatoid arthritis Syphilis Temporal arteritis	Cachexia Congestive heart failure Newborn status
Specimen handling	Refrigerated sample not returned to room temperature	Clotted blood sample Delay in testing
Technique	High room temperature Tilted ESR tube Vibration	Bubbles in ESR column Low room temperature Narrow ESR column diameter

From American Society for Clinical Pathology/American Proficiency Institute: 2006 2nd Test Event—Educational Commentary—The Erythrocyte Sedimentation Rate and Its Clinical Utility. API is the proficiency testing group that provides testing materials to the American Society for Clinical Pathology. The educational commentary itself is written by ASCP. This reference can also be accessed at: http://www.api-pt.com/Reference/Commentary/2006Bcoag.pdf. Accessed November 5, 2014.

E. Procedure (Wintobe)

PROCEDURE

1. Use fresh blood collected in EDTA anticoagulant. A minimum of 2 mL of whole blood is needed.
2. After mixing the blood thoroughly, fill a Pasteur pipette using a rubber pipette bulb.
3. Place the filled pipette into the Wintrobe tube until the tip reaches the bottom of the tube.
4. Carefully squeeze the bulb and expel the blood into the Wintrobe tube while pulling the Pasteur pipette up from the bottom of the tube. There must be steady, even pressure on the bulb to expel blood into the tube as well as continuous movement of the pipette up the tube to prevent the introduction of air bubbles into the column of blood.
5. Fill the Wintrobe tube to the 0 mark.
6. Place the tube into a Wintrobe rack (tube holder) and allow to stand undisturbed for 1 hour at room temperature. The rack must be perfectly level and placed in a draft-free room.
7. Record the number of millimeters the red blood cells have fallen. Read the tube from the bottom of the plasma meniscus

TABLE 14-3 Factors Affecting the Erythrocyte Sedimentation Rate (ESR)

Category	Increased ESR	Decreased ESR
Blood proteins and lipids	Hypercholesterolemia Hyperfibrinogenemia Hypergammaglobulinemia Hypoalbuminemia	Hyperalbuminemia Hyperglycemia Hypofibrinogenemia Hypogammaglobulinemia Increased bile salts Increased phospholipids
Red blood cells	Anemia Macrocytosis	Acanthocytosis Anisocytosis (marked) Hemoglobin C Microcytosis Polycythemia Sickle cells Spherocytosis Thalassemia
White blood cells	Leukemia	Leukocytosis (marked)
Drugs	Dextran Heparin Penicillamine Procainamide Theophylline Vitamin A	Adrenocorticotropic hormone (corticotropin) Cortisone Ethambutol Quinine Salicylates

F. Sources of Error

- Increased anticoagulant
- Use of sodium/potassiumoxalate and heparin
- Significant change in the temperature of the room
- Slight tilt of the pipette
- Blood specimens must be analyzed within 4 hours of collection if kept at room temperature
- Bubbles in the column of blood
- Clotted specimen
- Tubes must not be subjected tovibrations

G. Automated ESR

This is not yet widely acceptable since it cannot completely finish the stages of sedimentation. The only advantage is smaller blood sample is needed and uses shorter time.

- Ves-Matic System (Diesse, Inc., Hialeah, FL)- optoelectronic sensor which measures the change in opacity of a column of blood as sedimentation of blood progresses.
- Sedimat 15 (Polymedco, Cortlandt Manor, NY)- uses the principle of infrared measurement
- ESRSTATPLUS System (Hema Technologies, Lebanon, NJ)- based on centrifugation
-
  - **Westergren**
  - M <50yo = 0-15 mm
  - M >50yo = 0-20 mm
  - F <50yo = 0-20 mm
  - F >50yo = 0-30 mm
  - Children = 0-10 mm
  - **Wintrobe**
  - Male = 0-9 mm
  - Female = 0-20 mm
  - Children = 0-13 mm

- Thin or flat cells have an increased surface/volume ratio

II. OSMOTIC FRAGILITY TEST

- A measure of the ability of the RBC to take up fluid without lysing
- Employed to help diagnose different types of anemia, in which the physical properties of red cells are altered.

A. Principle

- Red cells suspended in hypotonic solution of sodium chloride take up water, swell, become spheroidal, and after reaching the critical volume, eventually burst.
- Cells that are thicker than normal have a decreased surface/volume ratio.

B. Materials

❖Materials:

- ✓ 12 Kahn tubes or ordinary test tubes
- ✓ Test tube rack
- ✓ 0.5% NaCl
- ✓ Distilled water
- ✓ Capillary pipette or Sahli pipette
- ✓ Anticoagulated blood (Heparinized blood)

PROCEDURE (SANFORD METHOD)

1. Arrange 12 tubes in a rack. Label it from number 25 up to number 14, starting from the left.
2. With the capillary or Sahli pipette, place in each tube the number of drops of stock 0.5% NaCl indicated by the number of the test tube.
   Note: Hold the pipette at the same angle to ensure uniformity of drops
3. With the same pipette, add into each tube the number of drops of distilled water required to bring the value in each tube to 25 drops.

TEST TUBE NUMBER	25	24	23	22	21	20	19	18	17	16	15	14
Drops of 0.5% NaCl	25	24	23	22	21	20	19	18	17	16	15	14
Drops of Distilled Water	0	1	2	3	4	5	6	7	8	9	10	11
% NaCl (Concentration of NaCl)	0.50%	0.48%	0.46%	0.44%	0.42%	0.40%	0.38%	0.36%	0.34%	0.32%	0.30%	0.28%

PROCEDURE (SANFORD METHOD)

4. Mix by lateral tapping
5. Draw blood into a Sahli-Hellige pipette up to 20 ul or 0.02 mL and dispense in each test tube

TEST TUBE NUMBER	25	24	23	22	21	20	19	18	17	16	15	14
Drops of 0.5% NaCl	25	24	23	22	21	20	19	18	17	16	15	14
Drops of Distilled Water	0	1	2	3	4	5	6	7	8	9	10	11
% NaCl (Concentration of NaCl)	0.50	0.48	0.46	0.44	0.42	0.40	0.38	0.36	0.34	0.32	0.30	0.28
Amount of blood	Add 20 uL or 0.02 mL to all tubes											

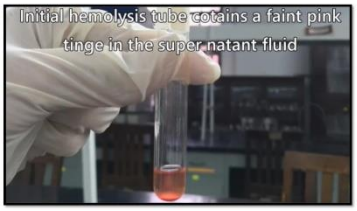
6. Mix by inverting the tube once or twice
7. Stand for two hours

D. Reference Value

Reference Value
initial = 0.42-0.44%
complete = 0.32-0.34%

DATA AND RESULTS

❖ Check for initial and complete hemolysis
❖ **Initial hemolysis**: tinge of pinkness in the supernatant and some cells at the bottom of the tube
❖ **Complete hemolysis**: clear, red supernatant with no microscopic cells at the bottom of the tube







Hemolysis = Tube number with initial and complete hemolysis x 0.02 ml (amount of blood in each tube)

EXAMPLE:

❖**Initial hemolysis:** Tube number 21 x 0.02 = 0.42%

- **Increased OFT** (decrease resistance): found in hemolytic anemias and hereditary spherocytosis and whenever spherocytes are found
- **Decreased OFT** (increase resistance): occurs following splenectomy and in liver disease, sickle cella nemia IDA and thalassemia

References:

- Rodak's Hematology: Clinical Principles and Applications, 5th Edition
- Clinical Hematology:Theory and Procedures, 5th Edition, Turgeon, M.L.
- Laboratory Sidenotes of Prof. Antonio C. Pascua,

WHITE BLOOD CELL COUNT
CLINICAL HEMATOLOGY 1

Instructor: Prof. Antonio C. Pascua, Jr., RMT, MSMT

Date: December 7 , 2021

Outline

At the end of the session, the student must be able to learn:

- **A. WHITE BLOOD CELL COUNT**
- **B. MATERIALS NEEDED (MANUAL)**
  - a. Hemacytometer
  - b. 1-3% Acetic Acid
  - c. 1 % HCL
  - d. Turk's diluting fluid
- **C. PROCEDURE**
- **D. POST LABORATORY**
- **E. SOURCES OF ERRORS**

I. WHTE BLOOD CELL

- The number of WBC's in 1 liter or 1microliter of blood (regardless if granulated or agranulated) WBC **count** accounts for all the white cells. In manual counting it is hard to identify which is which.
- Utilizes to indicate infection, during infection physiologically normal na yung wbc count is increased good sign since your body is helping you to eliminate infections.
- In manual white blood cell counting, you have to know the terms, leukocytosis and leukopenia, which is the elevated number of WBC above the normal range and the reduced number of WBC, respectively. People with leukopenia has higher risk of infection,.

II. MATERIALS NEEDED (MANUAL)

***COMPLICATED AND SUBJECTIV*

Hemacytometer
Thoma Pipet
Suction device
Thick coverslip
Cell Counter
Diluting fluids

1. HEMACYTOMETER

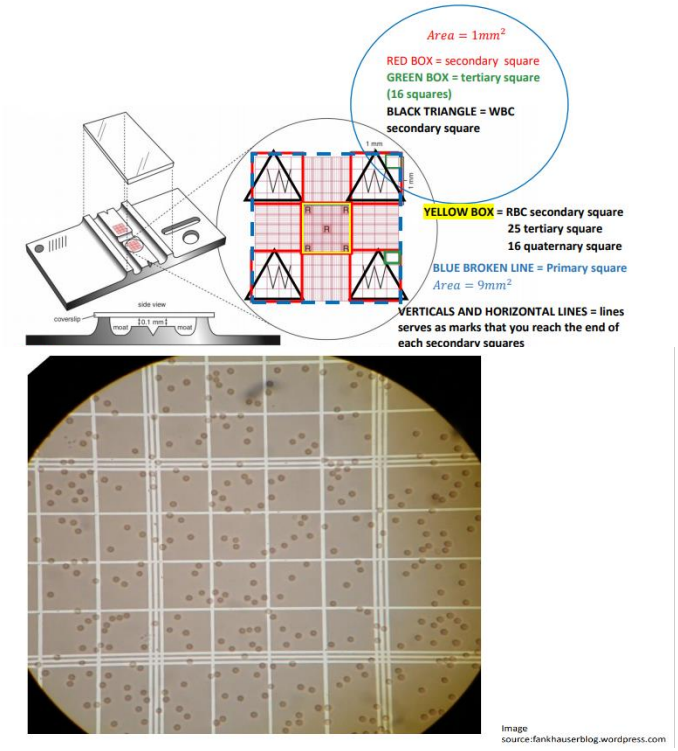
More convenient, accurate, organize

•"Heart of manual cell count"
•Different Types

- ***Improved Neubauer***
- Neubauer
- Fuchs –Rosenthal
- Speirs–Levy -Tuerk's
- Bass –Jones

*** The most commonly used counting chamber is the Levy chamber with improved Neubauer ruling.*

***Thoma pipette is also essential in manual cell counting procedures to accurately dilute blood specimens with fluids needed for specific cell counting procedures. A white cell pipet is the thoma pipet used in white cell counting. A red cell pipet can contain 101units of volume whereas white cell pipet can contain 11units.*



Primary square: 2
Each primary square has 9 SECONDARY SQUARE
The four corners of the secondary square is for WBC
WBC secondary square has 16 TERTIARY SQUARE.
For RBC in the center has 25 TERTIARY SQUARE
Each 25 TERTIARY SQUARE has 16 QUATERNARY SQUARE.
**each secondary square has 1mm x 1mm ➔ Total of $9mm^2$

2. WBC DILUTING FLUID

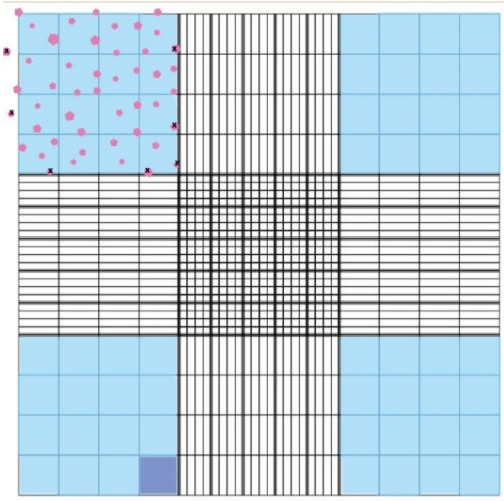
- 1-3% ACETIC ACID
- 1% HCl
- TURK'S DILUTING FLUID IS MADE UP OF:
  - Acetic acid
  - Distilled water
  - Gentian Violet Stain

  ****ISOTONIC solution (RBC)*
  ****HYPOTONIC (WBC) red cells must be lysed*

III. PROCEDURE

1. Using the WBC pipette, aspirate blood up to the 0.5 mark of the capillary stem of the pipette.
   NOTE: if blood is drawn too far above the 0.5 mark, the procedure should be repeated using a new pipette, excess blood causes an inaccurate result.
2. Wipe the outer walls of the pipette.
3. NOTE: Do not allow capillary attraction to draw fluid from the tip onto the gauze. Gauze tends to absorb the liquid portion of the blood, causing an inaccurate result.
4. Dip the pipette into diluting fluid, and then draw the diluting fluid into the pipette slowly, until the mixture reaches the 11 mark. Gently rotate the pipette to ensure a proper amount of mixing. The dilution is 1:20
5. Remove the tubing from the pipette, and then mix it in a horizontal axis for 5 minutes.
6. NOTE: To control the flow of the drops place the index finger on the bore to where the rubber tubing is fitted.

7. Discard the first 3-4 drops of the diluted sample.
8. Charge both sides of the hemacytometer counting chamber with a drop of the diluted sample and to stand for few minutes.
9. While keeping the hemacytometer in a horizontal position, place it on the microscope stage.
10. Count the white blood cells in the 4 'W' squares (FIG. 4). Using the L.P.O., the cells appear as r bodies with a definite outline. If doubtful whether an object is a leukocyte or artefact, keep on m the fine adjustment and take note of the well defined outline or better still examine under the H.P. verify.



USE L RULE, count all WBC's inside the 4 secondary squares

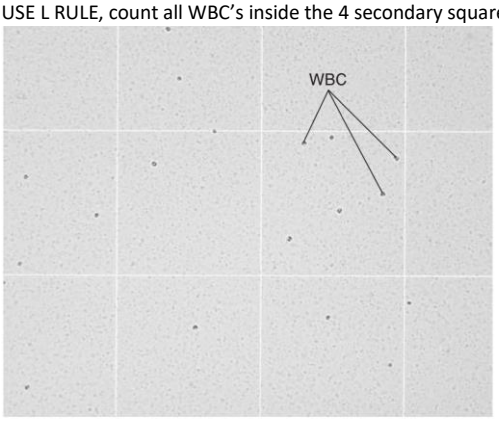


Figure 14-3 White blood cells as seen in the hemacytometer under low power (10× objective) 100× total magnification.

USE HPO to confirm the WBC

IV. POST LABORATORY

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor}}{\text{area (mm}^2\text{)} \times \text{depth (0.1)}}$$

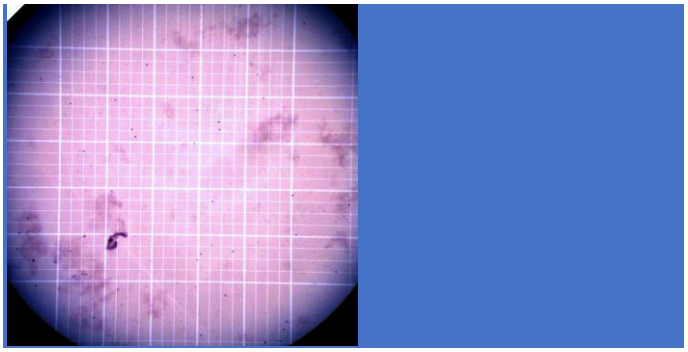
Or

$$\text{Total count} = \frac{\text{cells counted} \times \text{dilution factor} \times 10^*}{\text{area (mm}^2\text{)}}$$

EXAMPLE

$$\text{WBC count} = \frac{\text{cells counted} \times \text{dilution factor}}{\text{area counted (mm}^2\text{)} \times \text{depth}}$$
$$= \frac{96 \times 20}{4 \times 0.1}$$
$$= 4800/\text{mm}^3 \text{ or } 4800/\mu\text{L or}$$
$$4.8 \times 10^3/\mu\text{L or } 4.8 \times 10^9/\text{L}$$

Multiple to 0.001 to convert to the SI Unit



*****use of Turk's Diluting Fluid**

You may use this as a supplement reference in the WHITE BLOOD CELL COUNT Procedure

https://fankhauserblog.wordpress.com/1980/02/01/white-blood-cell-count/

V. SOURCES OF ERRORS AND COMMENTS

Dust and fingertips may cause difficulty in distinguishing the cells
Diluting fluid should be free ofcontaminants
Of the count is low, a greater area may be counted
Chamber must be charged properlyto ensure accurate count
Allow cell to settle for 10 mins before counting

***The accuracy of the manual WBC count can be assessed by performing a WBC estimate on a Wright-stained peripheral blood film made from the same specimen

N-RBC's

Falsely counted as WBC

Not lysed by WBC diluting fluids

Correct the WBC Count by dividing uncorrected WBC x100 by the number of nRBC per 100 WBC + 100

VI. NORMAL VALUES

- 4.0-11.0 x 10^9/L
- at birth: 10.0-30.0 x 10^9/L

Leukocytosis	Leukopenia
• Bacterial infection • Appendicitis • Leukemia • Pregnancy • Uremia • Ulcers ** in most cases sa infection, inc ang wbc. • Exudates: - Empyema / pyothorax or purulent pleuritis - Tuberculosis - Malignant neoplasms - Lymphoma - Mesothelioma - Pancreatitis - Rheumatoid arthritis	• Viral infections(HIV) • Brucellosis • Typhoid fever • Rheumatoid arthritis • Cirrhosis • Typhoid and paratyphoid • Anemia: - Aplastic anemia - Megaloblastic anemia • Hypersplenism • Drugs: - cytotoxic drugs • Radiation • Rarely leukemia • transudates: - Congestive heart failure - Cirrhosis with ascites

ADDITIONAL NOTES

- Whole blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) or blood from a skin puncture is diluted with 1% bufferedammonium oxalate or a weak acid solution (3% acetic acid or 1% hydrochloric acid).

- Alternately, a 1:100 dilution may be used counting the number of cells inthe entire counting area (9 large squares, 9 mm2) on both sides of the chamber (Table 14-1). As an example, if an average of 54 cells were counted in the entire counting area on both sides of the chamber

- Cell count:
  ** 1% ammonium oxalate. Df is 1:20,area counted is 4mm2
  ** 1% ammonium oxalate. Df is1:100, area counted is 9mm2

- Extremely elevated total leukocyte(WBC) counts of 50.0 × 109 /L or higher are consistent with a diagnosis of empyema.

- In general, WBC counts less than 1.0 × 109 /L are associated withtransudates, and WBC counts greater than 1,000 × 109 /L are associated with exudates.

OLFU
RMT 2023

WHITE BLOOD CELL DIFFERENTIAL COUNT

CLINICAL HEMATOLOGY 1

Instructor: Prof. Antonio C. Pascua, Jr., RMT, MSMT
Date: December 14 , 2021

LAB 9
TRANS 9

2021 – 2022
1st Semester
HEMA311
LAB

Outline

At the end of the session, the student must be able to learn:

- **A. LEUKOCYTE DIFFERENTIAL COUNT**
- **B. MATERIALS**
- **C. PROCEDURE IN SMEAR PREPARATION**
- **D. STAINING PROBLEMS**
- **E. DIFFERENTIAL COUNT**
- **F. ABSOLUTE COUNT**

A. LEUKOCYTE DIFFERENTIAL COUNT

- The white blood cell differential count determines the number of each type of white blood cell, present in the blood.

USES OF BLOOD SMEAR EXAMINATION

- Used for WBC Differential Count
- Can also count WBC but for estimation only
- Platelet estimation

Estimate means inaccurate value.

EDTA: specimen of choice because it preserves the cell morphology
OTHER SPECIMEN: skin puncture is also applicable but for small amounts of blood only.
FRESHLY COLLECTED BLOOD: within 2 hours, smears must be prepared.

PLATELET SATELLITISM: platelets adhere at the surface of neutrophils due to the used of EDTA and the antibodies may result in adhesion
CITRATE: specimen of choice for platelet counting.
Only used citrate if there is presence of satellitism

B. MATERIALS

- Cell counter
- Glass slides
- Microscope
- Cedar wood oil
- Spreader slides
- Wright's stain

C. PROCEDURE IN SMEAR PREPARATION

STEPS IN PREPARING SMEAR

1. After preparing the smear, fix it with methanol for 2-3 minutes
2. Allow to dry
3. Proceed with staining, flood the smear with Wright or Wright-Giemsastain for atleast 3 minutes
4. After 3 minutes, add the buffer.
5. Allow to buffer to stay for around 3 minutes
6. Mixing of the buffer and stain will produce green-metallic sheen
7. After staining, rinse the smear with neutral pH distilled water
8. Wipe the back of the slide. Air dry the slides
9. As drying the stained smear, make sure it is in vertical position

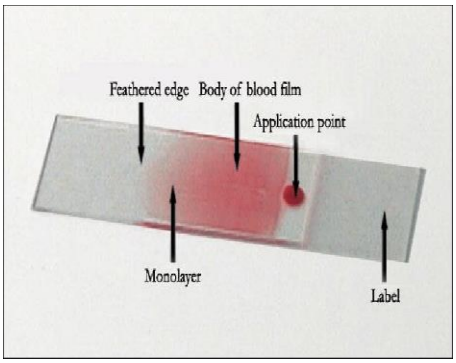
STAINING OF SMEARS

- Before staining, make sure that the slide is dry
- Stains:
  - Pure Wright Stain
  - Wright-Giemsa stain
- Purpose: make the cells more visible and to allow their morphology tobe evaluated
- Fixative: Methanol
- pH: 6.4 – 6.8; staining is always pH dependent

WEDGE TECHNIQUE: easiest way
Too much blood may result to thick smear
>3 mm in diameter: thick smear : falsely elevated

	THICK	THIN
Pressure	Decrease	increase
Angle (30 - 45 degree)	increase	decrease
Speed	Increase (short and dark red)	decrease
Specimen	>3mm increase	decrease

If there is presence of increase in Hct : thin smear should be prepared
THICK AREA: cells are clumping
THIN AREA: cells are deteriorated
THICK TO THIN AREA SMEAR TRANSITION: cells are equally distributed
As much as possible do not allow the end of the blood touch the sides of the slide



Acuña J, Largueza, J.M -- TRANSCRIBER

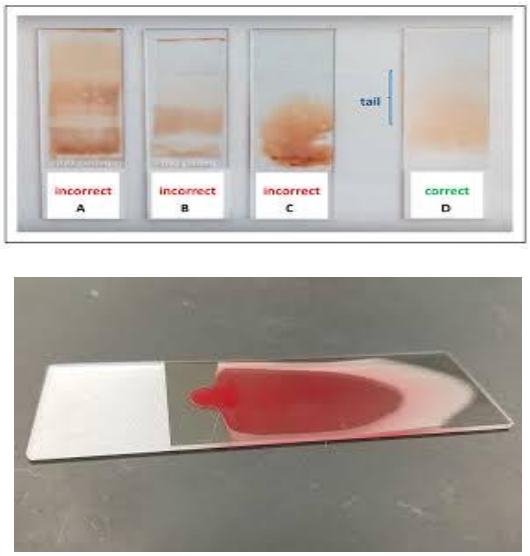


Figure 1-3: Examples of the Blood Smear

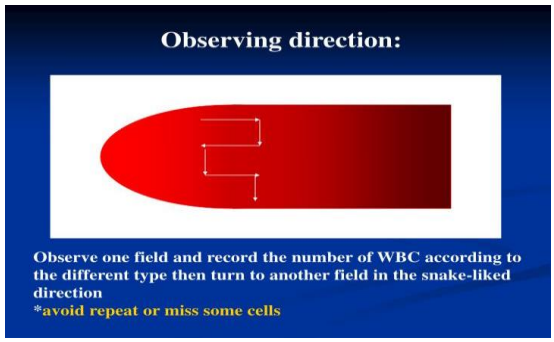
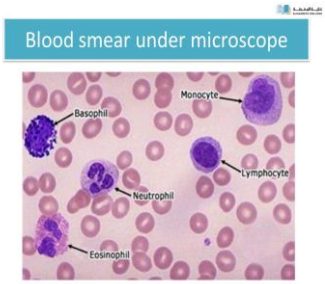
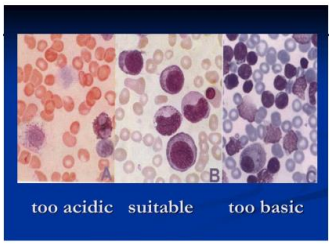


Figure 4: Observing direction of the blood smear.

BLOOD SMEARS UNDER MICROSCOPE





FEATURES OF A WELL-MADE WEDGE PERIPHERAL BLOOD FILM

1. The film is two thirds to three fourths the length of the slide
2. The film is finger shaped, very slightly rounded at the feather edge, notbullet shaped; this provides the widest area for examination
3. The lateral edges of the film are visible
4. The film is smooth without irregularities, holes or streaks
5. When the slide is held up to the light, the thin portion (feather edge) ofthe film has a “rainbow” appearance.
6. The whole drop of blood is picked up and spread.
7. There is a transition from thick to thin portion (area where cells areequally distributed

D. STAINING PROBLEMS

TOO ACIDIC STAIN	TOO ALKALINE STAIN
1. Thin blood smear 2. Insufficient staining time 3. Prolonged buffering or washing 4. Old stain	1. Thick blood smear 2. Prolonged staining 3. Insufficient washing 4. Alkaline pH of stain compone nts

MACROSCOPIC EXAMINATION

HEPARINIZED BLOOD SAMPLE: imparts bluish discoloration in smear background
GRAINY: RBC Agglutination
: Cold agglutinin: Anti-I Antibody

MICROSCOPIC EXAMINATION

Thick smear: overlapping of cells
High Power Objective (HPO) Field: specific area
: 10 HPO (white cells count estimation)

Computation
Average count cells x 2,000
SI unit : multiply to 0.001 x10^9/L

Oil Immersion Objective: used for white cells differential counting
: Platelet estimate: 10 OIO field

Computation
Average count x 20,000
Cells/uL x0.001 x10^9/L
Minimum white cell count must be 100 but due to some condition and hindi naabot yung 100, you can lower it down to 50
Battlement technique : zigzag like : performed it on the part where cells are equally distributed

E. DIFFERENTIAL COUNT

- One hundred WBCs are counted and classified through the use of push-down button counters
- Results are reported as percentage
- Principle: a stained smear is examined to determine the percentage ofeach type of leukocyte present and assess the erythrocyte and plateletmorphology.
- Manual : 100 WBC
- The more white cells counted, the more cells to be differentiated
- **Eosinopenia and Basopenia**: technically they **don’t exist** because **basophil and eosinophil are normal in low count**
- MANNER OF REPORTING : % (percent) : relative WBC differential count

TABLE 26.1 A Comparison of Normal Leukocytes in Peripheral Blood

	Segmented Neutrophil	Band Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Nuclear shape	Lobulated	Curved	Round	Indented or twisted	Lobulated	Lobulated
Chromatin	Very clumped	Moderately clumped	Smooth	Lacy	Very clumped	Very clumped
Cytoplasmic color	Pink	Blue, pink	Light blue	Gray-blue	Granulated	Granulated
Granules	Many	Many	Few or absent	Many	Many	Many
Color of granules	Pink, a few blue	Pink	Red	Dusty blue	Orange	Dark blue
Average percentage	56%	3%	34%	4%	2.7%	0.3%

Figure 5: Comparison of Normal WBC in the blood

F. ABSOLUTE COUNT

Computation
% relative count X WBC count

Unit: x10^9/L

***** Sum of absolute count must be equal to WBC count*
Scanner and LPO: coarse adjustment
OIO and HPO: fine adjustment

Addition of oil must be in between OIO and HPO objective (no need to bring down the film/stage

EXAMPLE ABSOLUTE BLOOD COUNT

- WBC absolute count:
  - WBC count = 6 x 10^9/L
  - Multiply the relative count with the WBC count:
  - Neutrophil: 50% x 6 = 3x10^9/L
  - Lymphocyte: 34% x 6 = 2.04x10^9/L
  - Monocyte: 10% x 6 = 0.6x10^9/L
  - Eosinophil: 5% x 6 = 0.3x10^9/L
  - Basophil: 1% x 6 = 0.06x10^9/L
- The sum of the values should and must be equal to the value of the WBC count

NOTE: The Trans might be different from the discussion of Sir Anton since no handouts was uploaded in the Canvas. Reading the book or other reference is highly recommended. Thank You and Happy Holidays