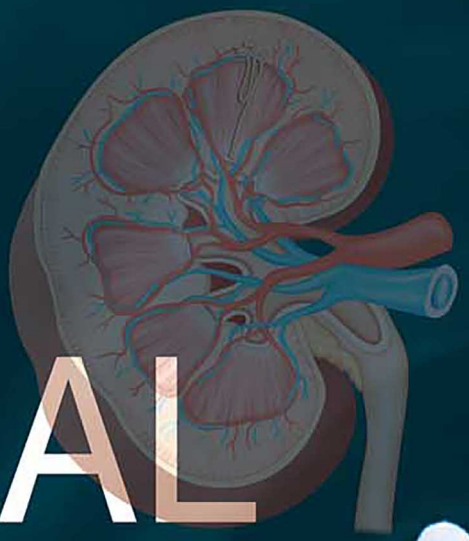
Ghai's Textbook of PRACTICAL PHYSIOLOGY



HIGHLIGHTS

- Updated important charts, questions, problems solving questions and calculations.
- Detailed objective structured practical examination (OSPE) for each practical.
- List of practicals requiring certification added.
- Richly illustrated.

As per the Competency-based Medical Education Curriculum (NMC)

Revised & Edited by
VP Varshney
Mona Bedi

10th Edition

Contents

Competency Table

Number	Competency The student should be able to:	Core (Y/N	Suggested Teaching Learning method	Suggested Assessment method	Chapter Number	Page Number
SECTION 1: Hematology						
PY2.11	Estimate Hb, RBC, TLC, RBC indices, DLC, Blood groups, BT/CT	Y	DOAP sessions	Practical/OSPE/ Viva voce	1.4, 1.5, 1.6, 1.7, 1.9, 1.10, 1.11	21, 27, 32, 40, 57, 60, 71
PY2.12	Describe test for ESR, Osmotic fragility, Hematocrit. Note the findings and interpret the test results, etc.	Y	Demonstration	Written/ Viva voce	1.8, 1.16	51, 90
PY2.13	Describe steps for reticulocyte and platelet count	Y	Demonstration sessions	Written/ Viva voce	1.12, 1.15	79, 88
SECTION 2: Human Experiments						
PY6.8	Demonstrate the correct technique to perform and interpret Spirometry	Y	DOAP sessions	Skill assessment/ Viva voce	2.2, 2.3	106, 115
PY11.14	Demonstrate basic life support in a simulated environment	Y	DOAP sessions	OSCE	2.4	119
PY5.16	Record arterial pulse tracing using finger plethysmography in a volunteer or simulated environment	N	DOAP sessions, Computer assisted learning methods	Practical/OSPE/ Viva voce	2.5	124
PY5.12	Record blood pressure and pulse at rest and in different grades of exercise and postures in a volunteer or simulated environment	Y	DOAP sessions	Practical/OSPE/ Viva voce	2.6, 2.7	127, 137
PY3.15	Demonstrate effect of mild, moderate and severe exercise and record changes in cardiorespiratory parameters	Y	DOAP sessions	Practical/OSPE/ Viva voce	2.8	140
PY5.13	Record and interpret normal ECG in a volunteer or simulated environment	Y	DOAP sessions	Practical/OSPE/ Viva voce	2.9	143
PY3.16	Demonstrate Harvard step test and describe the impact on induced physiologic parameters in a simulated environment	Y	DOAP sessions	Practical/OSPE/ Viva voce	2.10	151
PY10.20	Demonstrate (i) Testing of visual acuity, color and field of vision, and (ii) Hearing, (iii) Testing for smell, and (iv) Taste sensation in volunteer/simulated environment	Y	DOAP sessions	Skill assessment/ Viva voce	2.11, 2.13, 2.19, 2.20, 2.21, 2.22, 2.23, 2.24, 2.25	154, 158, 160, 163, 164, 169, 170, 171
PY10.12	Identify normal EEG forms	Y	Small group teaching	OSPE/Viva voce	2.26	172
PY9.9	Interpret a normal semen analysis report including (a) Sperm count, (b) Sperm morphology, and (c) Sperm motility, as per WHO guidelines and discuss the results	Y	Lecture, Small group discussion	OSPE/Viva voce	2.30	191

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Number	Competency The student should be able to:	Core (Y/N	Suggested Teaching Learning method	Suggested Assessment method	Chapter Number	Page Number
PY9.10	Discuss the physiological basis of various pregnancy tests	Y	Lecture, Small group discussion	Written/ Viva voce	2.31	193
PY9.6	Enumerate the contraceptive methods for male and female. Discuss their advantages and disadvantages	Y	Lecture, Small group discussion	Written/ Viva voce	2.32	195
SECTION 3: Clinical Examination						
PY11.13	Obtain history and perform general examination in the volunteer / simulated environment	Y	DOAP sessions	Skill assessment/ Viva voce	3.1	199
PY6.9	Demonstrate the correct clinical examination of the respiratory system in a normal volunteer or simulated environment	Y	DOAP sessions	Skill assessment/ Viva voce/OSCE	3.2	203
PY5.15	Demonstrate the correct clinical examination of the cardiovascular system in a normal volunteer or simulated environment	Y	DOAP sessions	Practical/OSPE/ Viva voce	3.3	209
PY4.10	Demonstrate the correct clinical examination of the abdomen in a normal volunteer or simulated environment	Y	DOAP session	Skill assessment/ Viva voce/OSCE	3.4	214
PY10.11	Demonstrate the correct clinical examination of the nervous system: Higher functions, sensory system, motor system, reflexes, cranial nerves in a normal volunteer or simulated environment	Y	DOAP sessions	Skill assessment/ Viva voce/OSCE	3.5	218
SECTION 4: Experimental Physiology						
PY3.18	Observe with computer assisted learning (i) amphibian nerve—muscle experiments, (ii) amphibian cardiac experiments	Y	Demonstration, Computer assisted learning methods	Practical/ Viva voce	4.1–4.18	249–295

1.5: THE TOTAL LEUKOCYTE COUNT

STUDENT OBJECTIVES

After completing this experiment, the student should be able to:

- Indicate the importance of doing total leukocyte count (TLC) in a clinical setting and in practical physiology.
- Do the total leukocyte count by the manual method, and compare its degree of error with the error of RBC counting.
- Name the constituents of Turk's fluid and their functions.
- Indicate the precautions you will observe.
- Describe the normal TLC in different age groups.
- Name the different leukocytes, their site of formation, functions, and regulation of leukopoiesis.
- Define leukocytosis, leukopenia, and physiological and pathological conditions in which they are seen.

INTRODUCTION

PY2.11: Estimate Hb, RBC, TLC, RBC indices, DLC, Blood groups, BT/CT.

The white blood corpuscles (WBCs, leukocytes) constitute the major defense system of the body against invasion by bacteria, viruses, fungi, toxins, and other foreign invaders. Their number is kept remarkably constant in health, but it increases or decreases in many diseases particularly acute and chronic infections. A clinician generally wants this test done along with differential count, hemoglobin, etc. as part of "complete blood count" (CBC) in cases of fever, especially if the cause of fever is not immediately apparent (pyrexia of unknown origin or PUO).

STAGES OF LEUKOPOIESIS (FLOWCHART 2)

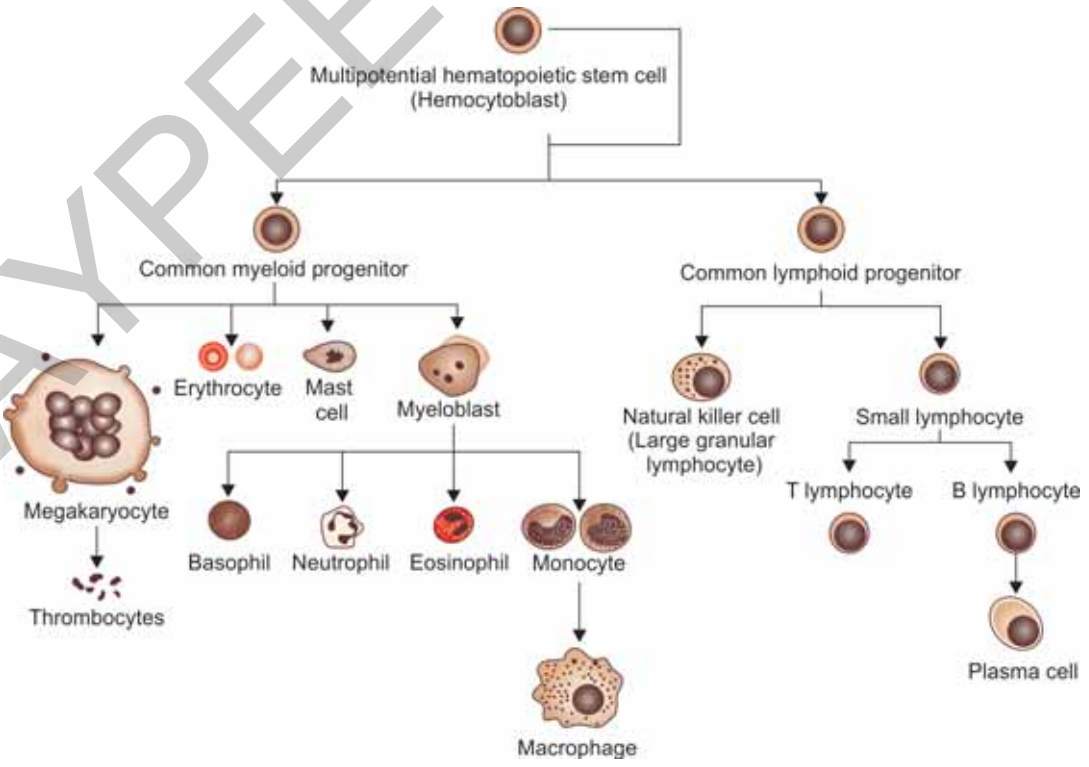
Normal count: 4,000–11,000 cells/mm^3 of blood in adults. The count after birth may be as high as 18,000–20,000/ mm^3, the normal levels being reached in a few years. In the adults, about 55–75% of the WBCs are granulocytes, while in young children, lymphocytes dominate. The count may be high in some physiological conditions such as heavy exercise, stress, etc.

TYPES OF LEUKOCYTES

The WBCs, unlike red cells contain nuclei but no hemoglobin. Depending on the presence or absence of clearly visible and conspicuous, chemical-filled granules (vesicles) in their cytoplasm (that are made visible by staining), they are grouped into two types: ***granular*** and ***agranular.***

- **Granulocytes:** There are three types of granulocytes that can be recognized under the compound microscope according to the coloration of their cytoplasmic granules—***neutrophils, eosinophils*** (eosin loving), ***and basophils*** (basic loving).
- **Agranulocytes:** In contrast to granulocytes whose nuclei are lobed, the nuclei of agranulocytes are not lobed but appear as a single mass. Although the cytoplasm contains chemical-filled granules, these are not visible under the light microscope due to their small size and poor staining with the usual dyes. The agranulocytes include—***monocytes*** and ***lymphocytes.***

FLOWCHART 2: Stages of leukopoiesis.



CHIEF FUNCTIONS OF LEUKOCYTES

- The chief function of leukocytes is to provide immunity (protection) against various invaders and thus constitute an important mechanism of survival by preserving health and fending off disease. The immune system consists of task-specific cells that are in a constant state of vigilance and readiness—like the branches of armed forces. They recognize the invaders as "foreign" to the body and engage them in combat at the site of invasion.
- The **tissue macrophages** (that develop from blood monocytes and act as the "sentinels"), and neutrophils (they act as the "infantry" and are transported by blood to the site of invasion), respond most quickly and destroy the invaders by phagocytosing them (both these cells move through the tissues by active amoeboid movements and are attracted to the inflamed area). Thus, these two types of WBCs along with antimicrobial proteins (interferons—alpha, beta, and gamma, and complement system and natural killer lymphocytes, form the ***second line of defense)***. ***The first line of defense*** against infection are the surface barriers i.e. intact skin and mucous membranes, that prevent the entry of pathogens into the body.

PRINCIPLE

A sample of blood is diluted with a diluting fluid which destroys the red cells and stains the nuclei of the leukocytes. The cells are then counted in a counting chamber and their number in undiluted blood reported as leukocytes/mm^3.

APPARATUS

- **Microscope:**
  - Counting chamber with a heavy coverslip
  - Blood lancet or pricking needle
  - Sterile cotton or gauze swabs
  - 70% alcohol.
- **WBC pipettes (Fig. 20):** White bead in bulb, and markings 0.5, 1.0, and 11. Two such clean and dry pipettes with free-rolling beads are required.
- **Turk's fluid:** This fluid is used for diluting the blood.
  - Glacial acetic acid = 3 mL. This destroys the membrane of RBCs, platelets and imparts refractility to the cells.
  - Gentian violet (1% solution) = 1.0 mL (it stains the nuclei of leukocytes).
  - Distilled water = To make the total volume to 100 mL.

Note: It would be impossible to count WBCs unless the RBCs were first destroyed. This is achieved by acetic acid (At this concentration the cell membranes of WBC's are not lysed). The dye gentian violet stains only the nuclei of leukocytes without staining their cytoplasm.

PROCEDURE

- Take 1 mL of Turk's fluid in a watch glass. Place the counting chamber on the microscope stage. Adjust the illumination and focus the right upper group of 16 WBC squares. You will see all the squares in one field.
- Observing all the aseptic precautions, get a fingerprick, discard the first two drops of blood, and let a good-sized drop to form.
- **Filling the pipette:** Dip the tip of the pipette in the edge of the drop, draw blood to the mark 0.5 and suck Turk's fluid to the mark 11. Mix the contents of the bulb thoroughly for 3–4 minutes.
- **Charging the chamber:** Discard the first two drops of fluid from the pipette so as to empty the fluid present in the stem. Charge the chamber on both sides. The chamber should neither be over-charged nor under-charged.
- Allow the cells to settle for 3–4 minutes, and then carefully transfer the chamber to the microscope. Use the fine adjustment again and try to identify the WBCs.

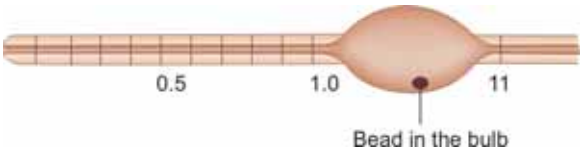


FIG. 20: The WBC pipette. It has 3 markings—0.5, 1.0, and 11.

Under low magnification: The leukocytes appear as round, shiny (refractile), darkish dots, with a halo around them. These "dots" represent the nuclei, which have been stained by gentian violet. The cytoplasm is not stained.

Important: When examining cells or counting them, do not keep a fixed focus but continuously "rack" the microscope so that the cells and the lines come into and go out of focus. In this way, you will not miss cells sticking to the undersurface of the coverslip, or confuse dust particles for WBCs.

Counting the cells: The procedure for counting the WBCs is similar to that employed for red cells **(Fig. 21)**.

- ❑ Count the cells under low power lens.
- ❑ You may count the WBCs in 16 squares under low power.
- ❑ Count the cells in the 4 groups of 16 square each, i.e., in a total of 64 squares.
- ❑ Draw appropriate squares in your workbook for entering the counts.

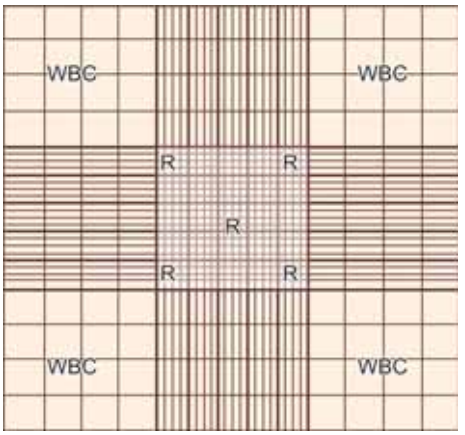


FIG. 21: Counting of cells.

OBSERVATIONS AND RESULTS

Calculations (Fig. 22)

Dilution factor = Final volume achieved (10 parts)/ Original volume taken (0.5 parts) = 20
Volume of fluid:
Area of 4 WBC squares = 4 × 1 × 1 = 4 mm^2
Depth of the chamber = 0.1 mm
Volume of fluid in the 4 WBC squares = 4 × 0.1= 0.4 microliter
Calculation of TLC:
Let N be the total number of WBCs in 4 WBC squares Total no. of WBCs in 1 microliter of undiluted blood = N × Dilution factor (20)/0.4 = N × 50

Sources and Degrees of Error

The sources of errors are the same as described for RBC counting and include: Pipette error, chamber error, field error, and experimental error.

The degree of error which may be 30% or more in RBC counting is much less in TLC (about 5–10%) because of the low dilution employed (1 in 20) in this case. The error can be further reduced if counting is done on both platforms of the counting chamber. That the error in TLC counting is much less important than that in the RBC count is obvious from the following example: In a TLC of 8,000/mm^3, even an error of 20% will give a count of 9,600/mm^3 which is again well within the normal range.

Note: In case when the leukocyte count is very high, as in leukemia, the dilution has to be increased. For this purpose, the RBC pipette can be used in which the blood is sucked up to mark 1 and diluted 100 times. Further calculation is done accordingly.

PRECAUTIONS

- Observe all precautions described for a finger prick, filling the pipette, and charging the counting chamber.
- Keep all the equipment ready before getting a prick.
- When mixing the blood with the Turk's fluid, give sufficient time for complete hemolysis of red cells. However, ensure that the leukocytes are not centrifuged toward the ends of the pipette which can be avoided by keeping the pipette horizontal while mixing the contents of the bulb.
- Continuously "rack" the microscope while identifying and counting the cells.
- Though the condition of a charged chamber may remain stable for 80–90 minutes, the count is usually stable for 30–40 minutes. After that, the diluted blood starts receding due to drying and the count decreases. The counting of the cells should, therefore, not be delayed.

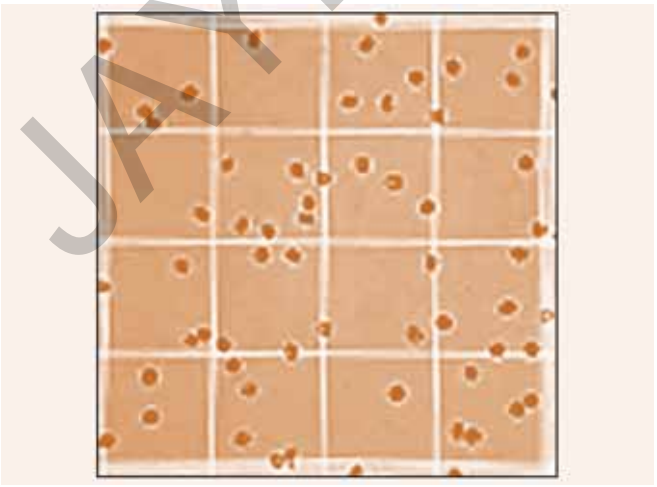


FIG. 22: White blood cells in WBC square.

PHYSIOCLINICAL SIGNIFICANCE

Conditions that increase TLC:

- **Physiological causes:** Physiological leukocytosis (i.e. in the absence of infection or tissue injury) has no clinical significance. There is no decrease or absence of eosinophils (eosinopenia) which is a feature of leukocytosis due to infection. Physiological leukocytosis is due to mobilization of WBCs from the marginal pool or bone marrow reserve ("Shift" leukocytosis). It is seen in the following conditions:
  - **Normal infants:** The count may be as high as 18–20,000/mm^3 but it returns to normal level within 1–2 years.
  - **Food intake and digestion ("digestive leukocytosis"):** There is a mild increase which returns to normal within an hour or so.
  - **Physical exercise**
  - **Mental stress**
  - **Pregnancy:** The count may be quite high especially during the first pregnancy.
  - **Parturition:** The high TLC is possibly due to tissue injury, pain, physical stress, and hemorrhage.
  - **Extremes of temperatures:** Exposure to sun, or to very low temperature can increase the WBC count.
- **Pathological causes:** A rise in TLC in disease is seen in:
  - **Acute infection with pyogenic (pus forming) bacteria:** The infection (due to cocci bacteria—*Streptococcus, Staphylococcus*) may be: (a) Localized, such as boils, abscess, tonsillitis, appendicitis, etc. (b) Generalized such as in septicemia and pyemia, bronchitis, pneumonia, peritonitis, meningitis, etc.
  - **Myocardial infarction:** The rise in TLC is due to tissue injury is not seen immediately after a heart attack but only after 4–5 days.
  - **Acute hemorrhage:** Maximum response occurs in 8–10 hours, the count returning to normal in 5–6 days.
  - **Burns:** Maximum response occurs in 5–15 hours, the count returning to normal in 2–3 days.
  - **Amebic hepatitis.**
  - **Malignancies:** High counts are seen in half the cases; secondary infection enhances the count.
  - **Surgical operations:** A postoperative rise is seen in all cases.

Conditions that decrease total leukocyte count:

- **Physiological causes:** A decrease in TLC under normal physiological conditions is unusual and rare. Exposure to acute extreme cold, may reduce the count to only slightly below the 4,000/mm^3 level.

- **Pathological causes:** Leukopenia is due to disease, where TLC is abnormally low is never beneficial to the body. In fact, it may endanger the life of the patient. The condition is almost always due to a decrease in neutrophils (neutropenia) and may be caused by various drugs used in treatment, radiation, or certain infections as described here:
  - **Infection with nonpyogenic organisms:** Typhoid and paratyphoid fevers, and sometimes in protozoal infection like malaria.
  - **Viral infections:** Influenza, mumps, smallpox, acquired immunodeficiency syndrome (AIDS).
  - **Drugs:** Chloramphenicol, sulfonamides, aspirin, penicillins, cyclosporins, phenytoin, etc. Cytotoxic drugs used in treating malignancies may also cause leukopenia by depressing the bone marrow (other blood cells may also decrease).
  - **Repeated exposures to X-rays and radium:** These are used as radiotherapy in cancers, and cause bone marrow depression.
  - **Chemical poisons that depress bone marrow:** Arsenic, dinitrophenol, antimony, and others.
  - **Malnutrition:** Deficiency of vitamin B_{12} and folate, general malnutrition, starvation, extreme weakness and debility.
  - **Hypoplasia and aplasia:** Partial or complete depression of bone marrow, i.e. failure of stem cells, may occur as a result of autoimmunity, and other factors.
- **Preleukemic stage of leukemias** may show leukopenia.

QUESTIONS

Q.1. How will you differentiate a WBC pipette from a RBC pipette?
See **Table 4**.

Q.2. What do the three markings on the pipette indicate? How do you get a dilution of 1 in 20?
See text above.

Q.3. What is the volume of the bulb in the WBC pipette? Why is its bulb smaller than that of the RBC pipette?
The volume of the fluid in the bulb is 10 times the volume of fluid in the stem, which can give a dilution of 1 in 10 or 1 in 20. In the RBC pipette, the volume of the bulb is 100 times the volume of the stem, which can give a dilution of 1 in 100 or 1 in 200. Since the count of leukocytes is in thousands/ mm^3, the blood requires much less dilution as compared to red cell count which is in millions/mm^3.

Q.4. What is the function of the bead in the bulb?
The bead serves three purposes:
1. It aids mixing the blood with the diluent.
2. It helps in identifying the pipette by just looking at it.
3. It tells whether the pipette is dry or not. In a dry pipette, the bead rolls freely without sticking to the inside of the bulb which would happen if the bulb were wet.

Q.5. What are the other uses of WBC pipette?
The WBC pipette can be used for diluting the blood for counting RBCs in cases of severe anemia, or for counting platelets. It can also be used for counting sperms and bacteria.

Q.6. What is the composition of Turk's fluid? What is the function of each constituent?
The diluting fluid for TLC contains glacial acetic acid, gentian violet, and distilled water. The acid hemolyzes the red cells without affecting the WBCs at this concentration. The dye stains the nuclei of leukocytes.

Q.7. What is meant by the term "glacial"? Why should the acid in the Turk's fluid be glacial?
The term glacial means pure acetic acid. Only the glacial acid can give the typical "shine" (halo) or clear refractility around the WBCs due to swelling of nuclei. This differentiates them from dust particles which are opaque and of different shapes (It is called glacial because during its manufacture, it gives the appearance of a glacier at one stage).

Q.8. Why are the red cells not seen when counting the leukocytes?
The red cells are not seen because they are hemolyzed by the acid (they would, otherwise, not allow counting of leukocytes). The remnants of red cell membranes are faintly visible—the so-called ghost cells.

Q.9. Can any other agent be used to hemolyze the red cells?
No. Any weaker hemolytic agent would take an inordinately long time to lyse them. A strong agent, on the other hand, in addition to lysing the red cells will also damage the leukocytes.

Q.10. What is the normal total leukocyte count?
See text above.

Q.11. What is the difference between differential leukocyte count and absolute leukocyte count?
In differential leukocyte count (DLC), the percentages of various types of WBCs are determined, while in absolute leukocyte count, the number of different WBCs per mm^3 is calculated (This is done from TLC and DLC).

Q.12. What are the various types of leukocytes and what are their functions?
See text above.

Q.13. What is leukopoiesis? Where does it occur?
See Flowchart 2 above.

The granulocytes, monocytes, and ***"lymphocyte precursors"*** are formed in the bone marrow (as are RBCs and platelets), while ***"blood lymphocytes"*** (those seen in blood films) are formed mainly in the ***"peripheral (secondary) lymphoid tissue"*** scattered throughout the body such as lymph glands, tonsils, spleen, Peyer's patches of intestinal mucosa, and the "lymphoid nests in the bone marrow".

Lymphocytes: All lymphocytes come originally from ***"bone marrow lymphocyte precursors",*** most of which are released into circulation though some remain in the bone marrow. Those that enter the blood are pre-processed cells. The processing occurs in either of the two central (primary) lymphoid tissues i.e thymus or bursa equivalent as shown in **Flowchart 1**.

Those that take up residence in ***thymus*** are programed by its environment into T-lymphocytes (T cells), while those that are processed ***in bursa equivalent tissues (fetal liver and bone marrow)*** become B lymphocytes (B cells) (In birds, the bursa of Fabricius, a lymphoid structure near cloaca is the site of pre-programming of B-lymphocytes).

After preprocessing in the central (or primary) lymphoid tissue, both T and B cells take up residence in ***peripheral (or***

secondary) lymphoid tissue. From these locations, various types of lymphocytes continue to divide and redivide and enter circulation throughout life via the lymphatic channels.

Thus, after birth, most lymphocytes are being formed in the peripheral lymphoid tissue, thymus and spleen, though some are formed in the bone marrow.

Granulocytes and ***monocytes*** are continuously formed in the red bone marrow to replace those millions of cells that leave the blood and enter the tissues, or those that are destroyed. Similarly, lymphocytes are being formed continuously to replace those lost.

Note: With the exception of lymphocytes, the other cells of blood do not divide once they leave bone marrow.

Q.14. How is leukopoiesis regulated?

Over 70 billion WBCs pass from the blood into the tissues every day, and the same number enters the circulation from their sites of production. In spite of these huge numbers involved, the constancy of TLC suggests a very efficient feedback mechanism that controls their production and release.

The substances which stimulate or inhibit this process appear to be many and varied. They include **colony stimulating factors CSFs** (formed by monocytes and T-lymphocytes), ***interleukins*** (formed by monocytes, macrophages, and endothelial cells), ***prostaglandins*** (formed by monocytes), ***lactoferrin,*** and possibly other agents. All these substances were collectively called ***Leukocyte promoting factor*** (or ***leukopoietin).*** Thus, unlike RBCs, the products of dead and dying cells themselves control leukopoiesis. The ***CSF,*** a glycoprotein present in the body fluids, appears to play an important role in the physiological regulation of leukopoiesis. During tissue injury and infection, the bacterial toxins, products of injury, etc. cause great increase in the rate of production and release of leukocytes.

Q.15. What is meant by the terms leukocytosis and granulocytosis? Name the physiological and pathological conditions which cause leukocytosis.

While *granulocytosis* technically refers to an increase in the number of granulocytes (neutrophils, eosinophils and basophils), *leukocytosis* refers to an increase in the number of all white blood cells beyond 11,000/mm^3. Leukocytosis is a normal, protective response of the body to various types of stresses such as infections, severe exercise, surgery, tissue injury, etc.

The TLC may rise due to:

- **Redistribution within the blood:** The WBCs from the "marginal" pool are mobilized and poured into the circulating blood.
- **Release from bone marrow reserve:** This is another process by which the number of WBCs can be raised in a short time.

These two processes raise the TLC without increasing their rate of production. There are no immature cells in the blood.

Note: It is important to remember that leukopenia due to any cause makes a person more likely to get pyogenic and other infections.

Q.16. What is leukemia and what are its major types?

A malignant progressive disease of hematopoietic cells leading to abnormal proliferation of immature or abnormal leukocytes is called leukemia. There is an uncontrolled production and release of mature and immature WBCs into the circulation. The leukemias (commonly called blood cancers) may be **myeloid** (usually involving neutrophils) or **lymphatic** (involving lymphocytes), and acute or chronic.

In acute leukemia, there is accumulation of immature cells in the blood (acute lymphatic leukemia is the most common malignancy in children, while acute myeloid leukemia is common in adults). Chronic leukemia begins more slowly and may remain undetected for months. Mature cells accumulate in blood because they do not die at the end of their normal lifespan.

In most cases, the cause is not known. However, genetic factors, viruses (e.g. human T cell leukemia, lymphoma virus-1, HTLV-1), chemical factors, and ionizing radiations (accidents in atomic power plants, atomic blasts such as in Hiroshima and Nagasaki during World War II) are involved.

Q.17. What is the difference between leukocytosis, leukostasis, leukemoid reaction, and leukemia?

Leukocytosis: It is an increase in TLC count above 11,000/mm^3, irrespective of the types of cells involved. It may be physiological or pathological. The pathological causes include infection and tissue injury. The count usually does not exceed 20–25,000/mm^3 and there are no immature cells in the circulation.

Leukostasis: If the count is more than 100,000/mm^3, white cell thrombi may form in the brain, lung, and heart—a condition called leukostasis. Transfusion of blood before TLC is reduced, increases blood viscosity, thus increasing the risk of leukostasis.

Leukemoid reaction: It is an extreme elevation of TLC above 50,000/mm^3 as a result of the presence of mature and/or immature neutrophils. The causes include: Severe chronic infections, especially in children, severe hemolysis, malignant growths (cancer of breast, lung, and kidney). It is not leukemia, and can be distinguished from chronic myelogenous leukemia (CML) by estimating the leukocyte alkaline phosphatase (LAP level which is elevated in leukemoid reaction, but depressed in CML).

Leukoerythroblastic reaction is similar to leukemoid reaction but with the addition of nucleated red cells (normoblasts) on blood smear. The causes include: Marrow infiltration by malignancy, hypoxia, and severe anemia.

Leukemia: Leukemia is a cancerous growth of blood forming organs (bone marrow or lymphatic tissues). Due to uncontrolled production, both immature and mature WBCs are released into circulation. The TLC is generally above 40–50,000/mm^3 or even a few lakhs. Even when the count is moderately high, it is not called leukocytosis. Most cells are, however, functionally incompetent.

Table 10: Classification of anemias on the basis of MCV and MCHC.

	Normochromic	*Hypochromic*
Normocytic	After acute hemorrhage	After chronic blood loss
	Hemolytic anemias, except thalassemias	—
	Renal disease	—
	Aplastic anemia, chronic infection	—
Microcytic	—	Iron deficiency anemia
	—	Thalassemias (due to globin deficiency)
	—	Hypoproteinemia
Macrocytic	Deficiency of vitamins B_{12} and folic acid	Secondary to liver disease

have more MCV and MCH but the saturation will not exceed 38%.

Q.4. How will you classify anemias according to their cause?

See chapter on hemoglobin.

Q.5. How will you classify anemias on the basis of MCV and MCHC?

The anemias can be classified on this basis as given in **Table 10**.

1.10: BLOOD GROUPING (BLOOD TYPING)—ABO AND RH SYSTEM

STUDENT OBJECTIVES

After completing this experiment, the student should be able to:

- Define the terms blood "groups" and "blood types", and name the various blood group systems.
- Describe the physiological basis of blood grouping and its clinical significance.
- Describe the Landsteiner's law and explain the basis of the terms "universal" donor and "universal recipient".
- Describe the Rh factor and how it was discovered? What is its significance?
- Determine blood groups by using commercially available antisera and precautions to be observed.
- Explain how blood is stored in blood banks, and the changes that occur in blood during storage.
- List the indications for blood transfusion and the dangers associated with it.
- Explain why is essential to match donor and recipient blood groups before giving a transfusion.
- Describe the effects of mismatched blood transfusion.

INTRODUCTION

PY2.11: Estimate Hb, RBC, TLC, RBC indices, DLC, Blood groups, BT/CT.

The surfaces of human red cells contain a variety of genetically determined glycolipids and glycoproteins that act as antigens. The plasma contains antibodies that can react with these antigens when the two are mixed. Since the red cell antigens cause agglutination of RBCs in the presence of suitable antibodies, they are also called ***agglutinogens;*** the antibodies in the plasma are called ***agglutinins.***

BLOOD GROUP SYSTEMS

1. **ABO Blood Group (Classical)**
2. **Rh Blood Group (Rhesus)**
3. **Others, e.g. M or N.**

A group of related red cell antigens that show similar chemical, genetic, and reactivity properties constitutes a ***"blood group system".*** Within a blood group (e.g. ABO system), there may be two or more different ***"blood types"*** (e.g. A, B, O, and AB). However, the terms blood groups and blood types are often used synonymously.

There are at least 30 commonly occurring antigens and hundreds of rare antigens that have been found on the surfaces of human red cell membranes. Of different blood group systems, only two are of great clinical importance. These are the ***ABO system*** and the ***Rh system.*** Other blood group systems are—MN, Lutheran, Kell, Colton, Duffy, Kid, Diego, Lewis, Li, Yt, Xg, P, C, etc. These groups are of little importance because they are not antigenic, though they are of value in anthropological and genetic studies. Some of these function as cell recognition molecules.

Most Common Blood Groups in India

- O+ = 32.53%
- O- = 2.03%
- A+ = 21.8%
- A- = 1.36%
- B+ = 32.09%
- B- = 2.01%
- AB+ = 7.70%
- AB- = 0.48%

Physiological Basis of ABO System

The antigens A and B are complex oligosaccharides differing in their terminal sugars. Those found on red cells are glycolipids, while those found in tissues and body fluids are soluble glycoproteins. The fucose-containing **H antigen is the basic antigen and is found in all individuals.** In the case of antigen A, a transferase places N-acetylgalactosamine as the terminal sugar on antigen H. In antigen B, the terminal sugar is galactose. In AB persons, both transferases are present, while group O individuals have none of the enzymes, so that the **antigen H persists in them.** Normally, H antigen has no antigenic activity and, as a result, it is identified by the

Table 11: Results of reaction of different blood groups with different antibodies.

Blood group	Antigen on cell membrane	Antibodies in serum	Reaction with		
			Anti-serum A	Anti-serum B	Anti-serum Rh
A	A	anti-B	–	+	
B	B	anti-A	+	–	
AB	No antigen	anti-A, anti-B	+	+	
O	A, B	No antibodies	–	–	
Rh+	Rh	No antibodies			+
Rh–	No Rh antigen	No antibodies			–

("+": **Agglutination present**—RBCs are massed together in clumps and lose their outline; "–": **No agglutination**—RBCs remain separate and evenly distributed)

capital letter O. Since H antigen is not antigenic, there are no corresponding antibodies. (It seems that group O persons produce a protein that has no transferase activity; this results from single base deletion in the corresponding gene).

Genetic Basis of ABO System

The blood group of a person is determined by two genes, one on each of two paired chromosomes.

These genes can be any one of three types—A, B, or O, but only one type is present on each of the two chromosomes. The O gene is functionless and does not produce O antigen on red cells, while A and B genes produce strong antigens on red cells. Thus, there are six possible combinations of genes—AA, BB, AB, OA, OB, and OO—and each person is one of these six genotypes. A person with genotype O has no antigen on red cells and so the blood group is O. A person with genotype AA or OA is blood group A, while genotype BB or OB is blood group B.

Agglutinins of ABO System

The antibodies in the plasma are gamma globulins. The agglutinin reacting with antigen A is called anti-A (or alpha, α) and reacting with B antigen is called anti-B (or beta, β). Blood group O has no antigens, but both anti-A and anti-B antibodies in the plasma. Blood group AB has both A and B antigens, but no antibodies. *The immune system forms antibodies against whichever ABO blood group antigens are not found on the individual's RBCs.* Thus, a group A individual will have anti-B antibodies and a group B individual will have anti-A antibodies. *These antibodies are present without any specific red cell antigenic stimulus.* For example, they are absent in a newborn; the ABO antibodies start appearing in the plasma by the age of 3–4 months due to cross-reactivity of ABO antigens present in naturally occurring bacteria, viruses, pollen, etc. present in the environment.

Rh Factor

In addition to antigens of the ABO system, the red cells of 80–85% of humans also contain an additional antigen, called Rh antigen (or Rh factor) **(Table 9)**. The Rh factor is so named because this antigen was discovered in the rhesus monkey by Landsteiner and Wiener in 1940. They injected red blood cells of rhesus monkey (the common Indian variety with red ischial callosities) into rabbits. The rabbit's immune system reacted by forming antibodies against rhesus red cells, and when the rabbit plasma was tested against human red cells, agglutination occurred in 80–85% of individuals.

Table 12: Differences between ABO and Rh antibodies.

ABO system antibodies	Rh antibodies
The antibodies anti-A and anti-B are of the larger IgM type. They cannot cross the placenta.	Rh antibodies are of the IgG type. They can easily cross the placenta.
These antibodies react best with the antigens at low temperatures of 5–20°C. They are, therefore, called "cold" antibodies.	The antigen–antibody reactions occur best at body temperature. Hence, they are called "warm" antibodies.
ABO incompatibility between a mother and her fetus rarely causes any problems.	Rh incompatibility between a mother and her fetus may cause serious complications.

(Ig: immunoglobulin)

Persons whose red cells contain this additional antigen are called **"Rh positive" (Rh +ve, Rh+)** while those who lack this antigen are called **"Rh negative" (Rh –ve, Rh–)**.

There are several varieties of Rh antigen—C, D, E, c, d, and e—but the *D antigen is the most common, and antigenically, the most potent.* Therefore, Rh +ve persons are also called RhD +ve and Rh –ve are called RhD –ve. The antibody of D antigen is called anti-D antibody (anti-Rh antibody). **However, there are no naturally occurring antibodies against Rh (D) antigen. The Rh (D) antigen is not present in body fluids and tissues, but only on red cells.** This antigen is a "warm" antigen and can cross the placenta easily. The differences between ABO and Rh antibodies are given in **Table 12**.

PRINCIPLE

The surface of the red cell membrane contains a variety of genetically determined antigens, called **iso-antigens** or **agglutinogens,** while the plasma contains antibodies **(agglutinins).** To determine the blood group of a person, his/her red cells are made to react with commercially available antisera containing known agglutinins. The slide is then examined under the microscope to detect the presence or absence of clumping and hemolysis (agglutination) of red cells, which occurs as a result of antigen–antibody reaction. There is a reciprocal relationship between antigens on the red blood cells and antibodies in the serum is known as Landsteiner's law.

The Landsteiner law (1900), which has two major components, states that:

1. If an agglutinogen is present on the red cells of an individual, the corresponding agglutinins must be absent in the plasma.
2. If an agglutinogen is absent in the red cells, the corresponding agglutinins must be present in the plasma.

Note: The exception to the second component of the law is that absence of Rh agglutinogen from the red cells in Rh –ve persons is not accompanied by the presence of anti-Rh agglutinins. Obviously, this component of the law was enunciated before the discovery of Rh factor in 1940 by Landsteiner and Wiener.

APPARATUS

1. Microscope, "glass dropper with a long nozzle", sterile blood lancet or needle, "sterile cotton/gauze swabs", alcohol, 5 mL test tube, toothpicks.
2. Clean, dry microscope slides. (A special porcelain tile with 12 depressions is available for this purpose and may be used in place of glass slides).
3. Normal saline (0.9%)
4. **Anti-A serum:** This contains monoclonal anti-A antibodies (against humans); these antibodies are also called anti-A or alpha (α) agglutinins. The anti-A serum can also be obtained from a person with blood group B.
5. **Anti-B serum:** This contains monoclonal anti-B antibodies (against humans); these antibodies are also called anti-B or beta (β) agglutinins. The anti-B serum can also be obtained from a person with blood group A.
6. **Anti-D (anti-Rh) serum:** This contains monoclonal anti-Rh (D) antibodies (against humans). These antibodies are also called anti-D agglutinins.

Note: The large volume requirements for high quality ABO and Rh(D) typing reagents can now be supplied by selected monoclonal antibodies.

Monoclonal antibodies are antibodies produced by a single clone of cells or cell line and consisting of identical antibody molecules. A monoclonal antibody is created by exposing a white blood cell to a particular antigen protein, which is then cloned to mass produce antibodies to target that antigen.

Note: The antisera are available commercially. For a quick identification, the anti-A serum is tinted blue, anti-B serum yellow, while the anti-D serum is colorless **(Fig. 34)**. The antibodies against Rh factor do not occur naturally.

Do not interchange the droppers provided with antisera bottles.

PROCEDURE

Preparation of red cell suspension: A suspension of red cells in saline should preferably be prepared and used instead of adding blood drops directly from the fingerpick to the antisera for the following reasons **(Fig. 36)**:

1. Dilution of blood permits easy detection of agglutination and hemolysis, if present. (Red cells in undiluted blood tend to form large rouleaux and masses. These may be difficult to disperse and may be mistaken for agglutination).
2. Plasma factors likely to interfere with agglutination are eliminated.

FIG. 34: Commercially available anti-A, anti-B, and anti-D serum.

Method: Put 2 mL of saline in a small (5 mL) test tube. Then get a finger pricked and allow a blood drop to form. Now place the pricked fingertip on top of the test tube and invert it. Mix the blood and saline by inverting the tube two or three times. A suspension of red cells is now ready.

Determination of Blood Groups

1. Mark the wells in blood group slides as A, B and D separately.
2. Put one drop of anti-A serum, anti-B serum and anti-D serum separately in the three wells marked as A, B and D on the blood group slide.
3. In addition, also take 1 drop of normal saline as control in a separate well.
4. Add a drop of red cell suspension drawn from the bottom of the test tube by a dropper on each of anti-A, anti-B and anti-D sera and one drop on the normal saline taken on the "control" side (The nozzle of the dropper should not touch any of the antisera) **(Figs. 35A and B)**.
   In this way, the red cells–saline mixture on the "control" side will act as a control to confirm agglutination or no agglutination on the corresponding test side **(Fig. 37)**.
5. Gently mix the antisera and red cells suspension, using three separate applicator sticks.
6. Wait for 8–10 minutes then inspect the three antisera—red cell mixtures and the "control" mixture, first with the naked eye to see whether agglutination (clumping of red cells) has taken place or not.
7. Then confirm under a low magnification microscope, comparing each "test mixture" with the "control mixture". **(Fig. 37)**
   - If there is no agglutination, the RBCs remain evenly distributed and separated from each other.

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VP Varshney MBBS MD has been working as Professor of Excellence and Head, Department of Physiology, (Faculty of Medical Sciences–Delhi University), Maulana Azad Medical College, New Delhi, India. He has worked as Director-Professor and Head, Department of Physiology, Maulana Azad Medical College, New Delhi. He has been teaching Physiology for about 35 years. He joined the college in 1978 as an undergraduate and went on to do postgraduation in the same college. He has worked in Lady Hardinge Medical College as faculty and later joined his Alma mater as faculty in 1994. He has published a significant number of papers in national and international journals of repute. Also, he has received a number of awards for his contribution to academics.

Mona Bedi MBBS MD has been working as Director-Professor, Department of Physiology, Maulana Azad Medical College, New Delhi, India. She has teaching experience of about 30 years in Physiology. She is an undergraduate from Lady Hardinge Medical College, New Delhi. She did her postgraduation from Maulana Azad Medical College in 1995 and joined as a faculty in the same college in year 2000. A dedicated teacher, she is very popular among her students. She has a keen interest in research and has published a lot of papers in national and international journals. She has also received many awards for her contribution to academics and research.

They have also published "Practical Physiology-A Student's Workbook" in 2019, which is being used as a practical manual for medical undergraduates all over India.

Printed in India

Available at all medical bookstores
or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
EMCA House, 23/23-B, Ansari Road,
Daryaganj, New Delhi - 110 002, INDIA
www.jaypeebrothers.com

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ISBN 978-93-5465-895-2